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STUDIES ON THE REACTIONS OF PILOBOLUS TO LIGHT STIMULI

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(WITH TWELVE FIGURES)

The present investigation concerns itself for the most part with the problems of simultaneous stimulation. It was undertaken with a view to settling some of the problems suggested in a former paper on the light reactions of *Pilobolus* by Miss ALLEN and myself (1). Some of the objections inherent in the methods used in the earlier work were eliminated in the experiments here reported, and a study was also made of the reaction of single sporangiophores toward the light.

The work was begun under Dr. R. A. HARPER at the University of Wisconsin and completed under Dr. G. J. PEIRCE of Leland Stanford Junior University. I wish to acknowledge my indebtedness for their criticisms and suggestions. I wish also to express my gratitude to Dr. D. H. CAMPBELL for his interest in my work and for his courtesy in extending all the privileges of his laboratory. I thank Miss RUTH F. ALLEN, who began the study of the reactions of a single sporangiophore of *Pilobolus* toward the light and furnished the data taken on the evenings of May 18 and 19, 1910.

The present study of simultaneous stimulation by light is on the question of directive influence, and the literature discussed will concern that phase of light effect. The early work was carried on with light and gravity as the stimuli. NOLL (4) in 1892 pub-

lished his theory of "heterogene induction." He reviewed the earlier authors on the subject and described their conflicting results. According to NOLL's theory, if an organism is subjected to two stimuli, one gives impetus to the other which carries out the reaction. One stimulus only is responded to. There is no resultant reaction toward the two stimuli. But when light and gravity work together, there is a change of geotonus due to light.

Recently GUTTENBERG (2) studied the simultaneous effect of light and gravity, using seedlings of *Avena sativa*, *Brassica Napus*, *Agrostemma Githago*, and *Helianthus*. He used what he called the compensation method. He tried different light intensities. With the higher intensities the reaction was toward the light alone. By gradually decreasing the intensities, GUTTENBERG found a certain light strength which just equaled that of gravity, and he obtained a resultant reaction between the two. A little weaker or a little stronger light gave a resultant reaction, varying according to the intensity of the light. GUTTENBERG considers this as evidence against NOLL's theory of "heterogene induction."

RICHTER (5), working along the same lines as GUTTENBERG, came to quite different conclusions. For his experiments RICHTER used *Avena sativa*, *Vicia sativa*, *Vicia villosa*, *Brassica Napus*, and *Helianthus*. He followed GUTTENBERG's method and in each case carried on a set of experiments in pure air and a similar one in impure air. He concludes that GUTTENBERG did not establish a resultant reaction between the effect of light and gravity by means of his compensation method, but that the latter's results were influenced by the impure air in which the experiments were performed. GUTTENBERG (3) followed this by a second paper in which he still maintained his former views. In this he repeated his own experiments, taking precautions to work under pure air conditions.

The experiments on simultaneous stimulation reported in this paper were performed with stimuli of the same kind, that is, they were light stimuli only. Before entering into a description of the work of simultaneous stimulation of *Pilobolus*, an account of some observations made on the reaction of a single sporangiophore of *Pilobolus* will be given.

Study of the reactions of the individual sporangiophore to a single light

In the earlier experiments (**2**), and also in those on simultaneous light stimulation in this paper, I was concerned with a large number of sporangiophores and with the net result of the reaction. This set of experiments was inaugurated for the purpose of following in detail the stages in the reaction of the individual sporangiophore toward light. The horizontal microscope was employed for this purpose.

A culture of *Pilobolus* in a 5-cm. flower pot was used. The pot was supported in an upright position. A thin glass Petri dish, measuring 5 cm. in diameter and 4 cm. in height, was placed over the top of the flower pot to keep the culture from drying. A 16-c.-p. carbon filament incandescent light was placed at a distance of 30 cm. from the culture, with the central point of the filaments 5 cm. above the level of the surface of the culture. The experiments were performed in the dark room and no other light had access to the culture. A horizontal microscope was placed with the tube on a level with the surface of the culture and at right angles to the direction of the light rays reaching the culture, so that any bending toward the light could be observed. A micrometer scale was placed in the ocular of the microscope in order to measure the change of position of the sporangiophore. In favorable cases several sporangiophores could be observed in the field of the microscope.

The first culture used in these experiments was put in place at 7:15 P.M. The sporangiophore had been exposed to the afternoon light and had grown straight out toward it, making an angle of 45° with the vertical. The culture was placed with the sporangiophores leaning away from the light, so that the angle between the light rays and the sporangiophore was about 135°. At the time when the experiment was set up, the young sporangiophores showed no signs of the sporangial swelling or vesicular bulb.

Two sporangiophores were observed during a period of 3 hrs. on the evening of May 18, 1910, and sketches were made at intervals during the reaction. The exact time when the reaction of the

first sporangiophore became perceptible was not determined. The reaction was distinctly visible at 7:45, 30 min. after it was exposed to the light. So far as could be seen, the curvature began at the tip of the sporangiophore, the tip bending as it grew. The tip distinctly started to curve upward at 7:45. This curve was somewhat more pronounced at 7:50. The tip was vertical, having moved through an angle of 45°. The radius of curvature was short. At 8:40 the tip had grown so that it was no longer vertical, but made a smaller angle with the incident light rays. At 9:30 the tip had grown around so that it pointed in the direction of the light. It had curved about 135° since the beginning of the observation. The curvature took place as the growth occurred; the curved end of the sporangiophore at this period was a well rounded hook. From

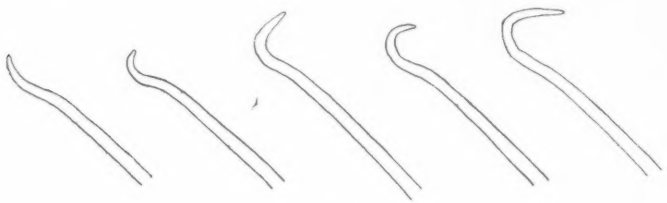

FIG. 1

this time on the tip grew straight toward the light. The last observation on this sporangiophore was made at 10 P.M. and showed a pronounced growth in the direction of the light.

Fig. 1 shows the stages that were sketched. The arrow indicates the direction of the light. The bending in this case had taken place always at the tip, the growing point of the sporangiophore. The older basal portion of the sporangiophore appeared to maintain the form and position which it had at the beginning of the experiment. If there was any change, it was so slight as not to be detected with the microscope.

The behavior of another sporangiophore under observation at the same time was as follows. The reaction was somewhat longer, no sign of curvature being noticeable until 8:00 P.M., 45 min. after the beginning of the experiment. The bending progressed slowly. At 8:40 the tip had curved through 45°, the curve being gradual.

At 9:15 the tip seemed to have stopped bending and to have grown straight. The angle made with the light was 60°. From 9:15 to 9:50 the increase in length was slight, and the older portion of the curve seemed to have become slightly more bent. At 10:15 the terminal sporangial swelling was well defined and the limits of the vesicular bulb could be discerned. The direction of the tip remained unaltered and formed an angle of 60° with the incident light rays (fig. 2).

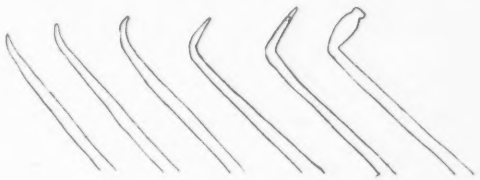

FIG. 2

A group of sporangiophores on the same culture as the two described above and subjected to the same stimulation from 7:15 P.M. until 9:55 were observed at 9:55. Three of them were still turned in the direction from which the afternoon light had come and away from the light used in the experiment. Apparently there was no response toward the light stimulus. In these three cases sporangium-formation had begun. The fourth sporangiophore had been subjected to the same conditions. The tip was curved through 135° and proceeded to grow in the direction of the light. The tip was still slender and pointed. The difference in length between this one and the other three was noteworthy. It exceeded the other three by the length of the portion beyond the bend where it turned toward the light. The sketches of the four sporangiophores as they were at 9:55 are shown in fig. 3.

FIG. 3

At 8:15, May 19, 1910, an older culture was used. Sporangium-formation had started when the observations were begun. At the beginning of the experiment the five that were chosen for study were at different stages, the youngest showing the sporangium as a

small yellow knob, and the oldest having its sporangium full grown and turning black. They were all pointed in the direction of the afternoon light, making an angle of 135° with the incident electrical light rays. The five were watched closely from 8:15 to 9:55 P.M., the position of the tip of each being observed and recorded every 5 min. During this time their development continued normally, the sporangia of the younger ones swelled, turned gray, and then nearly black, and the vesicular bulbs of all increased in size. In no one of the five was there any sign of bending toward the light, although they were watched for 1 hr. and 40 min.

On the evening of May 21, 1910, observations were made on the reactions of three sporangiophores. The light was turned on at 7:38. The sporangiophores were inclined away from the direction of the light at an angle of 125°. The sporangial swelling in all of them was yellow. The vesicular bulbs had not begun to form. At 8:02 the sporangiophores had grown 1 mm. in length, but there was no change of position due to the presence of the light. The observation was continued until 9:20 P.M. The sporangiophores had not reacted toward the light, although they had continued their normal development.

At 9:20 P.M., January 19, 1911, observations were begun on six sporangiophores. They were in the same field. The light was turned on at 7:20 P.M. and observations were made for 2 hrs. One of the sporangiophores stood vertically. It was slender tipped and showed no signs of sporangial swelling. At 7:50, after 30 min. exposure to the light, the tips showed a very slight curvature. The reaction then stopped and the tip began to swell slightly. When the light was turned off, the sporangial swelling was distinct.

The remaining five sporangiophores at 7:20 stood at an angle of 135° to the incident light rays. All showed sporangial swelling, but the vesicular bulb had not started to form. There were no indications of response toward the light in any of them, although the sporangiophores continued their normal development throughout the experiment. At the close of the experiment the swelling of the vesicular bulb on all of them was just visible. Fig. 4 shows a sketch of these sporangia as they appeared at the beginning of the experiment.

At 7:27, January 20, 1911, the light was turned on a culture which had been exposed to the afternoon light, and observations were begun on five sporangiophores which were visible in one field. The first sporangiophore made an angle of about 80° with the direction of the light. It was slender tipped. At 7:58 the tip showed a slight increase in length and this portion was very slightly curved toward the light. At 8:23 there was a slight increase in curvature toward the light. At 8:42 the tip had curved through 35°, making an angle of 45° with the light. At 8:50 the curvature had increased so that the angle between the direction of light and the tip was only 25°. At 9:30 the tip was pointed directly toward the light. From this time the tip grew directly toward the light (fig. 5).

FIG. 4

FIG. 5

The second sporangiophore observed was in much the same condition at the beginning of the experiment as the one just described, except that it was about 1 mm. longer. The reaction in this case was first noticeable at 8:00 P.M., the curve being barely visible. The curvature then proceeded more rapidly, and at 8:35 appeared more strongly than in the others in the field. At this time it had passed through an angle of 30°. At 8:42 the angle traversed was 45°. At 8:50 the angle made with the direction of the light rays was barely more than 10°, and at 9:13 the tip was directed straight toward the light and it continued in that direction until 9:50, when the observations were concluded. The portion beyond the curve was then about three-fourths of the length below (fig. 6).

FIG. 6

The third sporangiophore was inclined at an angle of 80° from the incident light rays and measured 4 mm. in length. It was slender tipped. Curvature was first visible at 7:58 and at 8:42 was still very slight. At 8:50 there had been a slight increase in length, although no further change in direction was noted. At 9:40 the curvature became more pronounced and at 9:50 the tip was pointing almost directly toward the light. At this time the curvature seemed to be arrested. No further observations were made on this sporangiophore (fig. 7).

FIG. 7

The fourth sporangiophore observed at the same time stood at an angle of 80° with the direction of the light. It was 2 mm. in length and the tip was slender and tapering. The reaction in this case was strikingly like that of the third sporangiophore just described. They were very near together (fig. 8).

The fifth sporangiophore measured 1 mm. in length at the beginning of the experiment. It was slender tipped and stood vertically from the surface of the culture. At 8:04 the tip had begun to curve. At 8:42 the tip had curved through an angle of 40°. At 9:13 it pointed in the direction of the light (fig. 9).

FIG. 8

Observations were begun on two sporangiophores on one culture at 7:40, January 21, 1911. They were slender tipped and made an angle of about 50° with the direction of the light. At 8:18 both showed new growth which was curved slightly toward the light. The curvature continued with the growth until at 8:50 the tip was directed straight toward the light. From that time until 9:50 when the observations were concluded, the sporangiophores grew in the direction of the light (fig. 10).

FIG. 9

A group of five sporangiophores was located in the field of the horizontal microscope at 8:40 P.M., November 2, 1911. The sporangiophores showed only slight differences in length and were inclined at an angle of 25° from the vertical. They were placed so that they leaned away from the light, making an angle of 95°. The sporangial swelling was just visible on the tips of all the spo-

rangiophores. They were observed continuously, but there was no sign of a bending toward the light until 3:00 A.M. Meanwhile, the sporangial swelling had increased in size and the vesicular bulb was barely visible. At this time the sporangiophores were curved slightly at some distance below the sporangium nearer the direction of the lamp. Observations were not made again until 4:30, when the sporangia were aimed directly toward the light and the vesicular bulb was distinct; the curvature was in the region immediately beneath the vesicular bulb. At 6:30, when the observations were concluded, the bulbs had swollen considerably. The sketches (fig. 11) show the development of one of the sporangiophores of the group. The development of all the others were remarkably parallel with the one described.

FIG. 10

Observations were begun using a second horizontal microscope on a sporangiophore at 9:00 P.M., November 2, 1911. The tip of the sporangiophore was just beginning to swell. The sporangium was standing vertical to the surface of the substratum. The light was placed at the angle above mentioned. The development of the sporangial swelling continued, but no reaction toward the light was visible at 11:35. Observations were made continually until 3:00 A.M., when the sporangium was rather well formed and the vesicular bulb barely visible.

FIG. 11

On the evening of November 3, 1911, two sporangia were observed for the first time at 10:40. The first sporangiophore was yellow and rather blunt tipped, but as yet it did not show sporangial swelling. It stood at an angle of about 20° from the vertical, thus being inclined away from the light at an angle of 90°. There was no sign of a response toward the light at 12:00 P.M., but the tip then showed the sporangial swelling. At 2:05 A.M. the sporangiophore began to curve toward the light, the region of curvature being then located at some distance below the sporangial swelling. At 2:15 the curvature was more pronounced but still confined to the

same region. The bending continued until 5:30 A.M., when the tip of the young sporangium pointed directly toward the light. The entire portion between the region of bending and the young sporangium was swollen slightly, showing the beginning of the formation of the vesicular bulb. At 6:30 the vesicular bulb was large and turgid (fig. 12).

At the beginning of the observation the second sporangiophore in the same field of the microscope was somewhat longer than the first and the sporangial swelling was well formed. This sporangiophore also formed an angle of 90° with the light. The vesicular bulb was not yet visible. At 2:15 the sporangiophores had curved through an angle of 20° nearer to the direction of the incident light rays. The curvature in this case was also at some distance below the sporangium swelling, and the space between the two was

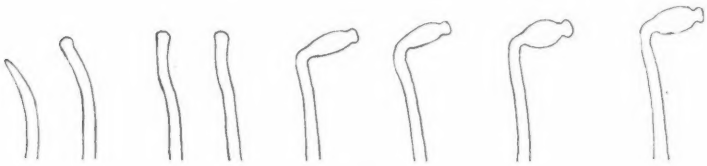

FIG. 12

beginning to show signs of the formation of the vesicular bulb. At 5:30 A.M. the tip of the sporangium was aimed toward the light. The vesicular bulb was still very inconspicuous. The bend in the sporangiophore was well rounded and had grown in length since the beginning of the curving. At 6:30 the vesicular bulb had swollen so that it exceeded the diameter of the sporangium by twice the diameter of the latter. At 8:00 the development appeared fairly complete. The sporangium was discharged between 9:50 and 10:00 A.M. (fig. 12).

From the foregoing experiments the following conclusions are evident:

1. Growth takes place at the tip in the young sporangiophore.
2. It is in the growing tip that heliotropic curvatures are formed.
3. In no case has a heliotropic curvature been observed during stages in early sporangium-formation.

4. Sporangium-formation may start during a reaction; in such cases the reaction is delayed for some time.

5. In case the reaction toward light is interrupted by sporangium-formation, it is resumed again a little before or about the time when the vesicular bulb is beginning to form.

6. After the sporangium is formed, the growing and curving portion is located immediately beneath the vesicular bulb.

The reaction of Pilobolus when exposed simultaneously to two equal sources of light

The effect of exposing a culture of *Pilobolus* simultaneously to two equal sources of white light was studied. For this purpose a light-proof box, measuring 120 cm. long by 60 cm. wide by 60 cm. high, was used. The box was made of pine and was painted a dull black on the inside. Running horizontally across one end, 20 cm. from the bottom, was an opening 10 cm. wide. Into the opening, which was prepared with rabbeted edges, was introduced a galvanized iron strip containing two openings 1 cm. in diameter and 9 cm. apart. The culture was placed at a distance of 25 cm. from the central point between the openings and on a level with them. It was placed with its surface vertical and facing the side of the box containing the openings.

The object of the experiment demanded that the light from the two openings be equal, but I know no methods of obtaining absolutely equal light intensities. We can only know that they are approximately equal. In order to obtain as nearly as possible equal illumination at the two openings, one light of measured intensity was placed equidistant from the two openings of the box and 40 cm. in front of it. Two mirrors were so adjusted that the culture intercepted the single spot of light formed by the convergence of the two sets of light rays. The light from either mirror was excluded from the opposite opening by means of the following device. At right angles to the edge of the box, along a line equidistant from the two openings, was placed an upright piece of board measuring 60 cm. high by 30 cm. wide by 1 cm. thick. At right angles to the first piece and parallel to the end of the box was nailed a second piece measuring 60 cm. high by 10 cm. wide by 5 mm. thick.

Both of the pieces were painted a dull black. The surface of the culture was covered with black paper, exposing a circular area 2 cm. in diameter. This small area was selected in order to exclude objectionable features such as unevenness in surface of culture, irregularity of distribution, etc. The number of sporangia was thus somewhat limited, but the undesirable features above mentioned were minimized.

This set of experiments was carried on in a dark room at the University of Wisconsin during April, May, and June 1910, under the direction of Dr. R. A. HARPER.

As previously described, a new set of sporangia matures daily and is discharged in the forenoon or early afternoon. The records of the results of the experiments were made daily in the late afternoon or evening. A glass plate fitting inside the box and against the openings caught the sporangia as they were discharged toward one or the other of the two lights.

The data were then recorded by means of a chart devised to meet the requirements of the experiment. The chart consisted of a large white sheet of paper divided by means of parallel lines into vertical strips 1 cm. wide. This is the principle of the Wolfhügel counter used by bacteriologists, and it was well adapted to the work in hand. The pieces of glass covering each of the 1-cm. openings fitted into the 1-cm. strips. In recording the data, the sporangia falling above and below the opening in the 1-cm. strip are recorded with those striking the opening. This is entirely fair, since, owing to the object of the experiments, we are concerned only with lateral distribution. Furthermore, our earlier experiments showed clearly the conditions of vertical distribution. The data for these experiments are recorded in table I.

In the first experiment 86 sporangia were discharged on the glass, 29 striking the vertical area containing the opening to the left and 25 that to the right. In the second experiment the total number was 60; 5 of these were on the area of the left opening and 20 on the right. In the third experiment 59 sporangia were counted on the glass, 5 and 18 being found on the left and right openings respectively. In the seventh all of the 22 sporangia discharged were fired toward the right opening, 10 of them striking the vertical

area containing the opening. Experiment 26 shows a total of 142 sporangia, 54 striking the vertical strip of the left opening, 30 that of the right opening. Experiment 27 shows 39 and 58 out of a total of 204 sporangia on the left and right openings respectively; and in experiment 28, 334 sporangia were discharged, 84 on the left and 78 on the right opening.

Throughout the series of experiments some of the sporangia failed to hit either opening. In experiment 1, 32 of the 86 sporangia discharged were of this sort. The distribution on either side of the two openings showed considerable variation. There were 9 sporangia in the 1-cm. strip to the left of the left opening and one sporangium in each of the next two strips. In the first 1-cm. strip to the right of the opening were 6 sporangia. On the left side of the right opening there were 11 sporangia in the first strip and 1 in the third; 3 sporangia were found in the first strip to the right of this opening.

In the second (table I), 3 sporangia struck the glass within 1 cm. to the left of the left opening. In the first, second, and third strips to the right of the opening were respectively 4, 2, and 1 sporangia. To the left of the right opening were 5 sporangia, all within the first strip. The number of sporangia in the first three consecutive strips to the right of the opening were respectively 18, 1, 1. The distribution and number of scattered sporangia in the remainder of the experiments showed about the same degree of variation as indicated by the complete data (table I).

In the foregoing experiments the number of sporangia which strike the openings does not appear especially large. These alone do not by any means give a complete conception of the accuracy of the total discharge of sporangia. A further knowledge of the distribution of these sporangia serves to correct the erroneous impression given by stating only the numbers that reach or do not reach the opening. The accuracy is very striking, for further consideration shows that 29 of the 32 sporangia that missed the opening in the first experiment were found within the 1-cm. strips on either side of the openings; only 3.5 per cent fell outside that area.

A perusal of the remaining data shows practically the same accuracy of discharge. The greater portion of the sporangia that

failed to strike the openings were found in the strips adjacent to the openings and only a very small percentage fell outside this area.

It is plain from these data that although there is much variation in the number of sporangia fired toward the two openings, the sporangia cluster about each of the openings and show no tendency to strike between the two. The sporangia are discharged, there-

TABLE I

Total									Opening									Opening						No. of experiments
86	...	...	...	...	...	1	1	9	29	6	...	...	...	...	1	...	11	25	3	...	...	...	...	1
60	...	...	...	...	...	...	...	3	5	4	2	1	...	...	...	...	5	20	18	1	1	...	...	2
59	...	...	...	...	...	...	1	5	5	3	...	...	...	...	...	3	10	18	10	2	1	1	...	3
56	...	...	...	...	...	...	...	1	2	1	...	...	...	2	2	5	14	14	13	2	...	...	...	4
25	...	...	...	...	...	...	...	3	8	1	2	...	1	...	1	...	1	7	...	...	...	1	...	5
41	...	...	...	...	...	...	...	2	9	4	2	...	...	2	1	1	4	10	4	...	1	1	...	6
22	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	2	4	10	4	...	1	...	...	7
35	...	...	...	...	...	...	...	4	7	1	1	1	...	1	...	4	5	7	3	...	1	...	...	8
45	...	...	...	1	1	...	...	5	13	3	3	...	...	1	...	1	1	11	5	1	...	...	...	9
36	...	...	...	...	...	...	...	5	7	2	...	...	...	1	1	2	1	12	3	...	1	1	...	10
54	...	...	...	...	...	...	...	6	3	3	...	1	...	...	2	1	6	21	7	3	1	...	...	11
29	...	...	...	1	...	...	1	5	3	1	2	...	...	...	...	...	3	13	...	...	...	...	...	12
24	...	...	...	...	...	...	...	2	4	2	1	...	1	1	...	...	...	11	2	...	...	...	...	13
24	...	...	...	...	...	...	...	1	9	3	...	...	...	...	...	...	1	8	...	1	...	...	...	14
14	...	...	...	...	...	...	...	1	4	...	...	...	...	...	...	1	3	4	1	...	...	...	...	15
24	...	...	...	...	...	...	1	3	7	2	...	...	...	...	1	1	3	6	...	1	...	...	...	16
20	...	...	...	...	...	...	...	2	3	3	...	...	...	...	1	2	2	3	...	3	...	...	...	17
12	...	...	...	...	...	...	...	...	4	...	...	2	...	...	1	...	1	2	2	...	...	...	...	18
20	...	...	...	...	...	...	...	1	6	3	...	1	1	...	...	1	5	1	1	...	...	...	...	19
7	...	...	...	...	...	...	...	...	2	2	...	...	...	...	1	...	...	2	...	...	...	...	...	20
35	...	...	...	...	...	...	1	3	10	5	2	...	...	...	...	3	4	7	...	...	...	...	...	21
27	...	...	...	...	...	...	...	2	8	2	4	...	...	...	...	2	3	5	1	...	...	...	...	22
14	...	...	...	...	...	...	...	...	5	1	1	...	...	...	...	...	...	4	3	...	...	...	...	23
22	...	...	...	...	...	...	...	1	4	3	...	...	2	...	...	...	...	7	5	...	...	...	...	24
34	...	...	...	...	...	...	...	6	12	5	...	...	...	1	...	...	2	6	1	1	...	...	...	25
142	...	...	...	...	1	...	4	16	54	17	1	1	...	1	...	...	10	30	6	1	...	...	...	26
204	...	...	...	...	2	3	4	23	39	9	6	3	4	1	1	7	20	58	20	4	...	...	...	27
334	...	...	...	1	1	2	3	24	84	23	5	4	2	6	3	9	34	78	39	8	2	3	3	28
133	...	...	...	1	...	...	2	8	43	12	2	...	...	1	3	6	16	32	4	2	...	1	...	29
178	...	...	...	1	2	1	8	26	52	28	6	1	1	2	1	2	16	21	8	1	...	...	1	30
146	...	...	...	...	1	...	4	12	25	8	4	2	2	...	3	4	27	44	7	...	1	...	1	31
24	...	...	...	...	...	...	1	2	2	1	...	1	1	1	...	...	4	9	1	1	...	...	...	32
252	...	1	2	3	2	3	5	25	25	16	8	5	4	5	5	12	22	44	33	9	9	8	6	33
61	...	...	...	...	...	2	2	5	2	5	3	4	2	1	1	4	7	13	4	3	2	1	...	34
30	...	...	...	1	2	1	1	3	2	2	...	...	1	3	4	2	3	2	2	1	...	...	...	35
176	...	...	...	1	2	5	17	15	9	6	...	3	...	8	13	25	20	19	13	5	8	4	3	36

fore, toward one or the other of the two lights. In other words, as shown by the earlier set of experiments, there is no resultant reaction due to the presence of the two lights. If there were, the sporangia would be for the most part between the two openings. It is clear, therefore, that this simple organism, when subjected simultaneously to two equal light stimuli, will respond to one of the two stimuli, to the complete exclusion of the other.

The sporangia, however, are not discharged in equal numbers toward the two openings. In the extreme case all might go to one or the other of the openings. It happened that in only one case (experiment 7, table I) did this occur, and then the total number was 22, the number being small and affording less chance for variation than would a larger number. The entire data thus show a great diversity of results as regards the number fired toward either opening. It is significant that the element of chance enters strongly into these results.

The fact that the sporangia do fire toward one or the other opening and in any degree of variation suggests further that although a large number of sporangiophores arise from one mycelium, they are not gregarious as regards physiological response toward light stimuli, that is, they act as separate individuals toward a light stimulus.

A further set of experiments was arranged to determine whether the sporangia to the right of the culture are discharged toward the right opening and those to the left of the culture to the left opening.

With the apparatus arranged as before, a second series of experiments was made with a thin plate of glass placed with its edge vertical to the middle line of the exposed surface of the culture. This glass would then intercept the sporangia discharged from the left side of the culture to the right opening and vice versa.

In the first experiment 2 sporangia struck each of the two openings, the number of sporangia in the consecutive 1-cm. areas to the left of the left opening are 8, 6, and 1; 3 sporangia were found on the 1-cm. area to the right of the opening. The first, second, and third 1-cm. areas to the left of the right opening contained respectively 1, 2, and 1 sporangia. The glass placed vertically at right angles to the surface of the culture received 7 sporangia on the left side and 15 on the right. The distribution of those on the left was as follows: 4 in the 1st cm. toward the culture, 2 in the 4th cm., and 1 in the 7th. The distribution of the sporangia on the right-hand side of the glass shows 7, 5, and 3 in the first 3 cm.

These data, together with those of the second and third experiments made in this series, are found in table II. These experiments tend to show that some of the sporangia on the right side of

the culture fire toward the left opening and vice versa. Some of this may be due to reflection from the glass.

TABLE II

The culture midway between and facing the two openings; a glass plate before the openings; a second glass plate at right angles to the surface of the culture. The data from the plates before the openings are given in the first part of the table; those from the plate at right angles to the surface of the culture are referred to by the marks *, †, and ‡.

Total								Opening									Opening									No. of experiment	Date
26					1	6	8	**2**	3				1	2	1	**2**									1*	6/15/'10 7:30 P.M.
7							1						2		1	**2**	1								2†	6/16/'10 7:35 P.M.
38							1	**4**	7	9		1		1		2	**7**	1	4	1					3‡	6/17/'10 7:20 P.M.

* Sporangia discharged on glass at right angles to surface of culture	4			2			1										Surface of glass to left of culture										
	7	5	3														"	"	"	"	right	"	"				
† Sporangia discharged on glass at right angles to surface of culture	6	3															"	"	"	"	left	"	"				
	2	1	2	1	1				1								"	"	"	"	right	"	"				
‡ Sporangia discharged on glass at right angles to surface of culture	1	1		2	1				1								"	"	"	"	left	"	"				
																	"	"	"	"	right	"	"				

The behavior of Pilobolus when given two equal sources of light but with the angle between the two sets of incident light rays varied

The box used for this set of experiments was made of redwood and measured 120 cm. long by 45 cm. wide and 35 cm. high. A hinged cover was so rabbeted as to be light-tight and was supplied with ice box catches in order to fasten it down tightly. At one end of the box, 14 cm. from the base, was an opening 9 cm. wide all the way across from side to side and so arranged with galvanized iron rabbets as to carry strips of galvanized iron 10 cm. wide which just closed the opening. These strips contained the openings through which the light was admitted to the culture. They were made of galvanized iron so that they would be thin enough to avoid shadows being cast by the rim of the openings. The openings were 1 cm. in diameter and their centers were the following distances apart: 1 cm., 2 cm., 3 cm., etc. The inner surface of the slide containing the opening was flush with that of the end of the

box, so that the glass plate on which the spores were caught fitted closely against the slide containing the openings. The openings not desired could be closed by means of two additional slides fitted in the groove from either end and outside the slide containing the openings. The inside of the box and of the strips was dull black.

The culture in this series was kept throughout the experiment at a distance of 23 cm. from the central point between the two openings. The surface of the culture was vertical and faced the slide containing the two openings. The exposed surface of the culture was 2 cm. in diameter. The light entering each of the openings was made to fall upon the exposed surface of the culture. The intensities of the light entering the two openings were equal or as nearly so as they could be made by measurement. The following device was followed in order to make the lights as nearly equal as possible. A single carbon filament incandescent lamp was placed at a distance midway between the two openings. By means of two mirrors placed at equal distances and equal angles one on either side of the lamp, the light was reflected through the opening to the exposed surface of the culture. In order to exclude the light of either mirror from the opposite opening a partition was set up in front of the box midway between the two openings, exactly similar to that used in the set of experiments last described.

With the change in the distance between the two openings it was necessary to change slightly the angles of the mirrors from the light in order that the spot of light reflected from the mirrors through the openings would strike the exposed surface of the culture. The condition of the experiments thus necessitated a slight change of light intensity from experiment to experiment, but the intensities of the light passing through the two openings at any one time were equal.

This set of experiments was performed in a dark room in the botanical laboratory of Leland Stanford Junior University.

In the first experiment, when the distance between the centers of the two openings was 5 cm., a total number of 38 sporangia were fired on the glass, 10 and 13 striking the left and right openings respectively. Only 2 of the 15 sporangia which failed to strike the opening were outside of the adjacent 1-cm. strips.

In the second experiment, with the center of the openings 4 cm. apart, 19 sporangia struck the glass; 10 were on the left opening, 7 on the right. Of the remaining 2 sporangia, 1 struck in each of the 1-cm. strips to the left and right of the left opening.

The third experiment shows a total discharge of 63 sporangia. Of that number 16 struck the left opening and 20 the right opening; 27 failed to reach either opening, but all of that number, excepting 3, were within 1 cm. of the opening.

When the distance between the openings was 14 cm., 50 sporangia were discharged, 15 striking each of the openings; 6 sporangia were counted within the first 1-cm. strip to the left of the left opening, and 1 in the third strip; 3 and 2 were within the first and second strip to the right of that opening; 5 sporangia struck within the 1-cm. strip to the left of the right opening, and 2 and 1 in the first and second strips on the right-hand side.

With a distance of 27 cm. between the two openings, 200 sporangia were discharged on the glass, 61 and 37 striking the respective openings to the left and right. The majority of scattered sporangia are again grouped around the two openings. The data for these experiments are tabulated in complete form in table III.

The results of the experiments in which the distances between the openings were varied agree with those obtained in the preceding set of experiments, where the openings were kept at the same distance throughout the set of experiments. The sporangia were fired with great accuracy toward one or the other of the two openings. The distribution of the sporangia about the openings varied in about the same degree as in the case just mentioned; and in the same way, the sporangia which are outside the vertical strips containing the openings are mostly within 1 cm. of one of the openings.

The reaction of Pilobolus when stimulated simultaneously by lights of different wave-length

The problems connected with the simultaneous stimulation of an organism by light rays of various wave-lengths offer an interesting field to the investigator. But I know of no way of accurately comparing lights of different colors as to the total amounts of

TABLE III

Distance between center of openings equals number of spaces between heavy figures plus one space.

| Total | | |

radiant energy. The following experiments, therefore, are qualitative only; but I hope they will prove suggestive.

The different colors of the spectrum are represented in different proportions in the various incandescent lamps. These filaments are of standard make and the energy of the bulbs is measured in candle-powers. Thus by using bulbs of equal candle-power and current of known intensity we shall have somewhat comparable quantities.

Although this method is not all that could be desired, it has a very marked advantage over the colored solutions used by SACHS, and also the monochromatic glass plates that are so generally used in work on the effects of rays of various wave-lengths. The colored solutions absorb a large portion of the total energy emanating from the source, different solutions differing in this respect. In the case of the solutions (and it is also true of the colored plates) neither the intensity nor the energy of the lights can be compared. The incandescent lamps offer at least the advantage that they are of nominal commercial value; and with the advent of the knowledge of methods of comparison of intensities of colored lights, some exact idea of the intensities in the different parts of their spectrum may be obtained. Some study has been made to determine the distribution of the different wave-lengths in the different incandescent lamps, but so far it is insufficient for the present instance. It is known, however, that of the three incandescent lamps used in the experiments, the tungsten has the largest proportion of the actinic rays, the tantalum next, and the carbon least. The results with these filaments may thus serve to check up with those of the earlier work, in which the solutions and the plates of colored glass were used.

Experiments were made in order to test the relative efficiencies of different incandescent lamps in bringing about the reaction of *Pilobolus*. In these experiments the carbon filament, the tantalum, and the tungsten were compared. The experiments were performed in the dark room, using the redwood light-proof box already described. The openings were 1 cm. in diameter and the distance between them from center to center was 10 cm. The culture was placed 23 cm. from the point midway between the two openings.

The entire surface of the culture, which was 5 cm. in diameter, was exposed. Before each of the openings, at equal distances, was placed one of the two lights to be tested. The angle at which the lamps were placed was such that the spot of light from each lamp struck symmetrically the open surface of the culture.

The series of experiments was begun with a 32-candle-power carbon filament lamp before one opening and a 20-watt tungsten before the other. The first experiment gave a total of 92 sporangia; 31 sporangia were discharged toward the carbon filament and 61 toward the tungsten. In the second experiment 163 sporangia were discharged; 68 toward the carbon filament and 95 toward the tungsten. In the third experiment 387 out of a total of 784 sporangia went to the tungsten. Of the 1116 fired in the fourth experiment, 113 went to the carbon, 1003 to the tungsten. The next experiment shows a total discharge of 228 sporangia; 78 toward the carbon, 150 toward the tungsten. A new carbon lamp was put in the place of the old in the seventh experiment, but again a very much larger percentage was aimed toward the tungsten; 589 sporangia were fired, the ratio standing 168 toward the carbon as against 431 toward the tungsten. The data for this set of experiments are given in complete form in table IV. From this table a comparison of the accuracy of aim of *Pilobolus* toward the carbon and tungsten lamps can be made.

TABLE IV

Total	32-c.-p. carbon filament								20-watt tungsten							
92	...	...	...	...	...	...	6	**19**	6	...	...	...	...	...	3	7	**42**	9	...	...	...	...	...
163	...	...	...	...	...	1	3	**47**	14	2	1	...	...	1	1	3	15	**54**	20	...	...	1	...	...
784	...	1	1	...	...	6	64	**203**	86	15	6	4	2	2	5	3	40	**253**	88	3	...	1	1	...
1116	...	...	...	...	...	3	19	**69**	19	3	...	2	2	3	5	17	170	**607**	179	18	...	...	...	...
228	...	...	...	...	...	...	15	**47**	12	4	...	...	...	...	4	6	22	**101**	17	...	...	...	...	...
894	...	...	...	...	1	...	39	**111**	21	7	1	1	...	2	2	9	58	**503**	136	4	1	...	...	...
589	...	...	...	...	1	7	48	**78**	24	3	4	2	3	6	8	8	69	**258**	63	4	1	2	...	...

Of the 31 sporangia discharged toward the carbon lamp in the first experiment, 19 (61 per cent) struck the glass over the 1-cm. vertical strip containing the opening. Of the 61 sporangia

discharged toward the tungsten lamp, 49 (80 per cent) struck within the corresponding strip.

In the second experiment, of the 163 sporangia discharged, 68 were discharged toward the carbon filament, 47 (69 per cent) striking the 1-cm. strip over the opening; 95 were discharged toward the tungsten, 54 (56 per cent) on the strip over the opening. In this experiment, unlike the first, the larger percentage struck the strip over the opening in the case of the carbon filament.

In the third experiment, 387 sporangia were discharged toward the carbon, 203 (51.7 per cent) of them in the vertical strip containing the opening; 397 were discharged toward the tungsten, 253 (65.2 per cent) on the region of the opening.

The fourth experiment shows 69 sporangia, which is 61 per cent of the 113 sporangia discharged, toward the carbon, on the 1-cm. strip containing the opening; and 607 (61 per cent) of the 1003 sporangia fired toward the tungsten on the same region.

The remaining experiments of this series all show greater accuracy in the tungsten light than in the carbon filament light. With one exception, that of the second experiment, the discharge of the sporangia is more accurate toward the 20-watt tungsten used than toward the 32-candle-power carbon filament lamp, although the energy of the tungsten lamp is only half that of the carbon lamp.

Again, the percentages which strike the openings probably do not at first glance appear remarkable, but on noting in the first experiment that the 12 sporangia that did not strike the opening in the case of those fired toward the carbon light were all within 1 cm. of it, the accuracy is striking. Of the 19 sporangia which failed to strike the opening before the tungsten lamp, 16 were within 1 cm. of the opening and the remaining 3 were within 2 cm. of the opening.

In the second experiment, 21 of the sporangia fired toward the carbon light missed the vertical strip containing the opening; 17 of that number were within 1 cm. distance of the strip. Of the 41 sporangia that missed the opening in the case of those fired toward the tungsten, 35 struck within 1 cm. of the opening.

In the remaining experiments, most of the sporangia which failed to reach the opening in the strips fell within the first 1-cm.

strip on either side of them (table IV), showing the remarkable precision with which the sporangium is thrown toward a light.

A series of experiments was made to test the relative efficiencies of a tungsten and a tantalum lamp in bringing about a reaction of *Pilobolus;* 40-watt lamps of each kind were used. The tungsten was placed before the left opening, the tantalum before the right opening at a distance of 40 cm.

TABLE V

Total								Tungsten 40-watt									Tantalum 40-watt							
373	...	1	...	1	...	11	50	137	56	25	10	1	3	3	2	6	13	31	17	5	1	...	...	...
628	...	...	...	...	14	15	100	243	100	32	5	4	5	4	5	10	34	34	17	3	3	...	...	...
206	...	...	...	...	...	...	24	71	13	4	1	3	4	5	3	4	18	39	15	2	...	...	...	...
321	...	...	...	...	...	7	36	104	42	5	...	1	...	...	2	3	40	61	10	2	...	...	...	...
185	...	...	...	...	...	9	25	61	23	6	5	...	1	2	1	4	15	24	7	2	...	...	...	...
166	...	...	...	2	1	8	26	67	25	5	2	1	...	...	...	1	9	13	3	1	...	2	...	...
688	...	...	...	...	3	20	112	291	102	19	6	2	2	2	2	5	29	47	24	...	...	...	...	...
886	...	...	...	...	...	50	176	363	158	51	20	11	4	2	3	1	14	22	9	1	1	...	...	...
574	...	...	...	3	5	15	69	248	53	18	2	1	...	...	1	5	24	43	23	2	1	1	...	...
1091	...	...	...	6	20	46	156	354	164	46	21	8	7	8	13	20	40	141	51	15	5	...	...	...
404	...	1	1	5	2	21	81	166	71	29	17	3	2	3	3	8	7	27	9	4	2	2	...	...
367	...	...	...	...	1	17	82	143	28	25	15	7	1	...	1	4	8	27	7	1	...	...	...	...

In the first experiment, 373 sporangia were discharged on the glass, 294 toward the tungsten and 79 toward the tantalum. The second experiment showed a total number of 628 sporangia, with 516 and 112 toward the tungsten and tantalum respectively. The number of sporangia discharged in the third experiment was 206; of these, 118 were fired toward the tungsten and 88 toward the tantalum. The total number of sporangia in the fourth experiment was 321, 197 and 124 being fired toward the tungsten and tantalum. In the fifth experiment, 185 were discharged, 130 toward the tungsten and 55 toward the tantalum. The sixth experiment also showed a larger number had been fired toward the tungsten. The data for this set of experiments are found in table V.

The accuracy of aim toward the tungsten and the tantalum lamps was compared as in the preceding experiments. In the first experiment 46.6 per cent were discharged toward the tungsten, 39.2 per cent toward the tantalum. In the second experiment the

percentages fired toward the tungsten and tantalum were respectively 47 and 33.5. The third experiment shows 60.1 per cent before the tungsten and 44.3 before the tantalum; the fourth, 52.7 and 49.1 respectively; the fifth, 46.9 and 43.6; and the sixth, 48.9 and 44.8. The tenth and twelfth experiments show slightly larger percentages on the opening before the tantalum. The percentages figured out for the numbers striking either opening are given in table VI. The larger percentages strike the opening before the tungsten in these experiments. Most of the sporangia missing the opening strips in these experiments were found in the adjacent 1-cm. strips, as was the case in the foregoing experiments.

TABLE VI

Number of experiment	Percentages before tungsten lamp	Percentages before tantalum lamp	Number of experiment	Percentages before tungsten lamp	Percentages before tantalum lamp
1	46.6	39.2	7	44.3	42.7
2	47	33.5	8	41.3	40
3	60.1	44.3	9	59.5	43
4	52.7	49.1	10	42.9	47.6
5	46.9	43.6	11	41.7	40.9
6	48.9	44.8	12	44.2	56.7

In the next series of experiments a 40-watt tantalum was placed before one opening and a 20-watt tungsten before the other. The total number of sporangia fired in the first experiment was 1004; 731 were on the half of the glass toward the tantalum and 273 are on the half toward the tungsten.

The second experiment shows a total discharge of 407 sporangia, 329 on the tantalum as against 78 on the tungsten. The third and fourth experiments both show larger numbers on the tantalum. The data for this set of experiments are found in table VII. The accuracy of aim toward the two lights may also be obtained from the data in this table. Of the 731 sporangia discharged toward the tantalum lamp in the first experiment, 420 (54 per cent) struck the 1-cm. strip containing the opening. Of the 273 fired toward the tungsten, 149 (54 per cent) were on the corresponding strip. In the second experiment, the number of sporangia fired toward the tantalum lamp was 329; of these 161 (49 per cent) were in line with the opening. The total toward the tungsten was 78, with 37

(47 per cent) on the opening strip. In the third experiment, the ratio of the percentages of those on the 1-cm. strip containing the opening, in the case of the tantalum lamp, to that on the corresponding strip in the case of the tungsten is as 57 to 61. In the fourth experiment the accuracy of aim is very nearly the same toward the two lamps, although a greater proportion of the total number favor the tantalum lamp. The comparison of a 40-watt tantalum lamp against a 20-watt tungsten shows that a larger number of sporangia are discharged toward the tantalum, but that there is very little difference as regards the accuracy of aim toward the two lamps.

As in the set of experiments last described, a close examination of the data (table VII) reveals the fact that most of the sporangia that missed the vertical strips containing the openings were found within 1 cm. of them. An average of 7.8 per cent struck the glass more than 1 cm. laterally from the opening.

TABLE VII

Total								40-watt tantalum									20-watt tungsten						
1004	...	...	...	...	4	9	137	**420**	110	31	11	5	10	5	3	12	40	**149**	55	3	...	...	...	...
407	...	...	...	...	...	3	89	**161**	49	12	7	5	5	5	3	3	15	**37**	13	...	...	...	...	...
125	...	...	...	...	...	...	18	**40**	10	2	...	...	...	...	...	...	11	**34**	10	...	...	...	...	...
292	...	...	...	1	...	4	35	**111**	34	10	3	...	1	1	4	5	23	**45**	15	...	...	...	...	...

A 40-watt tantalum lamp was next compared with a 32-candle-power carbon filament with the results shown in table VIII. The first experiment gave a total of 96 sporangia, 36 of which were discharged toward the tantalum and 60 toward the carbon. The second experiment gave a total of 231 sporangia, with 164 fired toward the tantalum and 67 toward the carbon. In the third experiment 365 sporangia struck the glass, 268 over the tantalum and 96 over the carbon. In the fourth experiment, of the 157 sporangia fired, 98 were on the glass over the tantalum and 59 over the carbon. The fifth experiment showed a total discharge of 1039, 674 being found on the glass before the tantalum, 465 before the carbon. In the remainder of the experiments, as can be seen from

the data (table VIII), there is a larger percentage of sporangia over the tantalum. Thus, with the exception of the first experiment, the number discharged in the direction of the tantalum lamp surpassed that discharged toward the carbon.

TABLE VIII

Total								Tantalum 40-watt										Carbon 32 candle-power						
96	....	....	....	....	....	....	8	23	5	....	....	....	....	....	....	1	10	40	9	....	....	....	....	....
231	....	....	....	....	....	....	43	79	34	6	1	1	....	....	3	2	17	32	13	....	....	....	....	....
365	....	....	....	....	....	....	65	136	49	15	3	1	....	....	....	1	28	48	14	5	....	....	....	....
157	....	....	....	....	....	....	24	57	14	2	1	....	....	....	....	1	6	40	10	1	....	1	....	....
1039	....	....	....	....	....	7	116	323	107	15	3	2	2	2	1	15	91	242	105	8	....	....	....	....
76	....	....	....	....	1	2	12	25	1	1	3	....	....	....	....	2	6	17	6	....	....	....	....	....
404	....	....	....	....	....	1	35	164	29	1	....	....	....	2	1	1	26	110	31	3	....	....	....	....
1775	1	1	1	2	3	36	164	748	170	31	9	6	1	5	11	26	106	283	135	20	10	5	....	1
100	....	....	....	....	....	....	7	47	11	1	....	....	....	....	....	1	6	23	4	....	....	....	....	....
507	....	....	....	....	1	5	56	154	59	5	2	1	2	1	1	5	51	121	40	3	....	....	....	....
1442	....	....	....	1	3	8	208	553	246	24	8	2	2	7	6	28	85	183	63	6	2	3	....	2
813	5/11	4	4	10	15	20	58	178	77	15	15	17	16	18	25	31	65	93	71	18	18	9	5	11/4
174	....	....	....	....	1	3	29	44	8	2	1	1	1	3	2	7	15	30	22	7	....	....	....	....
249	....	....	....	1	....	3	20	95	18	1	1	....	....	....	1	3	30	58	17	1	....	....	....	....
111	....	....	....	....	....	....	11	69	17	1	....	....	....	....	....	....	2	9	2	....	....	....	....	....
104	....	....	....	....	....	....	10	61	17	1	....	....	....	....	....	....	3	10	2	....	....	....	....	....
184	....	....	....	....	....	....	19	101	19	1	....	....	....	....	....	....	7	28	9	....	....	....	....	....
194	....	....	....	....	....	....	22	62	27	1	....	....	....	....	....	....	6	55	20	1	....	....	....	....
123	....	....	....	....	....	....	12	35	8	....	....	....	....	....	....	2	10	42	14	....	....	1	1	....
1231	....	....	....	2	14	40	118	266	162	40	18	2	2	1	2	50	122	257	107	42	9	3	....	1
44	....	....	....	....	....	....	5	19	4	1	....	1	....	....	....	1	1	11	1	....	....	....	....	....
43	....	....	....	....	....	1	5	16	....	....	....	....	....	....	1	....	2	12	6	....	....	....	....	....
561	....	....	....	....	....	6	98	146	35	5	5	5	2	1	2	8	56	122	44	7	....	....	....	....

Of the 36 sporangia discharged in the direction of the tantalum lamp in the first experiment, 23 (63.8 per cent) struck the glass within the 1-cm. strip containing the opening. Of the 60 sporangia fired toward the carbon, 40 (66.6 per cent) struck within the 1-cm. strip containing the opening. In the second experiment, 79 (48.2 per cent) of the 164 sporangia that are discharged toward the tantalum are found in the 1-cm. strip over the opening; 32 (48 per cent) of the 67 discharged toward the carbon are on the corresponding strip. In the third experiment the percentages which strike the glass on the 1-cm. vertical strip over the opening before the tantalum and carbon filament lamps are respectively 50.7 and 50. In the fourth experiment 58 per cent of the sporangia fired toward the tantalum and 67.7 per cent of those fired toward the carbon filament lamp strike the strip containing the respective openings.

The above percentages striking the area of the 1-cm. opening before each of the two lamps compared are given, together with those computed for the remaining experiments, in table IX. The complete data from which the percentages were figured are found in table VIII.

TABLE IX

PERCENTAGES OF THE TOTAL NUMBER OF SPORANGIA DISCHARGED TOWARD THE TANTALUM AND CARBON FILAMENT LAMPS WHICH STRUCK THE 1-CM. VERTICAL STRIPS COVERING THE OPENING IN EACH CASE IN EXPERIMENTS 1–23 IN TABLE VIII

Number of experiment	Percentage before tantalum lamp	Percentage before carbon lamp	Number of experiment	Percentage before tantalum lamp	Percentage before carbon lamp
1	63.8	66.6	13	49.3	34.8
2	48.2	48	14	68.3	52.7
3	50.7	50	15	60.2	69.2
4	58	67.7	16	68.5	66.7
5	68.1	52	17	78.5	63.1
6	55.5	58	18	55.3	67
7	71.2	63.2	19	63.6	61.8
8	63.8	47	20	32.6	43.1
9	71.2	67.6	21	63.3	78.5
10	57	54.2	22	72.7	52.3
11	52.4	47.6	23	45.6	50.4
12	41.7	24.2	Average	58.6	55.9

In this set of experiments the accuracy of discharge varies considerably. In some cases the discharge toward the tantalum is more accurate; in others that toward the carbon lamp is more so. On the whole, however, the discharge is a little more accurate toward the tantalum, but the difference is so small that it is practically negligible.

From the percentages in table IX, it stands out clearly that wherever there was a small percentage which struck the opening before one lamp, there was usually a comparatively small percentage on the corresponding area before the second light. In the first experiment, 63.8 and 66.6 were the percentages striking the two openings. In the second experiment, 48.2 and 48 per cent struck the openings. In some cases there is less uniformity. The eighth experiment shows 63.8 per cent on one opening and 47 per cent on the other. But on the whole, a small percentage on

one opening is usually accompanied by a corresponding percentage on the other. It might be suggested that this may be due to the general condition of the culture at the time of the experiment. The accuracy might be affected by food supply, moisture, temperature, and other factors of importance to the physiological condition of the sporangiophores at the time of stimulation.

Here, as in the previous experiments, the sporangia that strike outside of the opening are to be found for the most part in the first vertical strips to the left and right of the openings. An examination of the data (table VIII) will show a good proportion of the cases where all that were fired toward a single light are found on the opening or adjacent strips. Thus it is clear that the accuracy is much greater than it would appear from an examination of table IX alone.

Summary

Physiologists, in studying the reactions of plants to stimuli, have for the most part worked with phototactic organisms or organisms of considerable complexity, individuals in which there was a differentiation of tissues, where the cells in one portion of the body may receive a stimulus, another perceive it, and still another respond to it. Such a study has the disadvantage of dealing with too many factors and accompanying phenomena. In *Pilobolus* the reaction is marked and can be easily studied. A single cell receives the stimulus and responds to it. The protoplasm of the cell receives the stimulus, perceives it, and reacts. The accuracy of response of *Pilobolus* toward the light is remarkable, when we consider its size and the distance through which it throws its sporangia. The sporangiophore scarcely ever exceeds 1 cm. in length, and is usually somewhat shorter, while the distance through which it discharges the sporangium in most of the experiments is over 25 times that measurement. The accuracy of response and the nicety of organization of such a mechanism can well be appreciated from the study of such experiments. From such work the capacities of a single cell can best be realized.

The results of the experiments in which *Pilobolus* is stimulated simultaneously by two lights bear directly on NOLL'S (4) theory

of "heterogene induction." According to NOLL, the reaction of an organism to one of two stimuli excludes the effect of the second stimulus. His work was concerned with two very different kinds of stimuli, light and gravity. The reaction to the stimulus of light excluded any response to the stimulus of gravity. Recent workers, GUTTENBERG (2) and RICHTER (5), have interested themselves along the same lines. GUTTENBERG maintains that if the light stimulus be diminished sufficiently, a resultant reaction between light and gravity will occur; that NOLL and the earlier workers had used light that was too intense. To RICHTER'S (5) criticism that his results were due to impure air, GUTTENBERG (3) responded by further work under improved conditions and reached essentially the same results as before.

In experiments on simultaneous stimulation of *Pilobolus* by lights of the same kind or of different kinds, we are dealing, unlike either of the foregoing cases, with simultaneous stimuli of one kind, namely, light alone. It was possible to have the stimuli at least approximately equal, and it was possible to have the arrangement such that neither source of stimulation had any advantage over the other. Further, the organism worked with was a simple one, the reaction concerning only a single cell. And the net result of the reaction was shown so plainly in the distribution of the discharged sporangia that it seems impossible that any indefiniteness or uncertainty could be entertained as to the reaction. The sporangia clustered always about one or the other of the two sources of illumination. There was no sign of a resultant reaction. Even if the individual sporangiophore did not receive equal illumination from the two openings, if there were any resultant reaction, it would be expected that the sporangium would be found in a position between the two lights, depending on the ratio of their intensities, differences of composition, and the like. Thus, it would be expected that all of the sporangiophores would be located between the two sources of illumination. Such a condition did not obtain in any case where the two light stimuli were used, whether or not they were the same as far as distribution in the spectrum was concerned. The sporangiophore reacted to one or the other of the two stimuli. The results obtained at least suggest that one stimulus does not

affect the reaction to the other. To this degree the results are corroborative of NOLL's theory. But NOLL's theory, based on his work with light and gravity as stimuli, suggests a change of geotonus due to the presence of the light. In working with the two light stimuli, the reaction to one of the two stimuli to the exclusion of the other cannot be explained in this way, although here, as above, the plant is subjected to two directive influences.

Where the two simultaneous stimuli were of different kinds, gravity and light, NOLL believed that light may call forth certain changes in the plasma which, directly or indirectly, cause the reaction. He says that perception and reaction may rest on entirely different characters; but the reaction may be carried out by the same changes. In work where the two simultaneous stimuli are of the same character, as in the present experiments, the reaction is less complicated. Thus but two sets of changes of the plasma need be concerned with the response.

The above experiments show that there is no sign of a resultant reaction, but that they can in no way determine whether, so far as perception is concerned, there is any influence of one light on the other. According to NOLL's theory, a change of geotonus takes place, due to stimulation by light; that is, there is a change of sensibility to gravity as such due to the perception of light. The question arises whether, with the presence of two light stimuli of the same kind acting through the same time, there is a corresponding change of sensibility toward one light owing to the mere presence of a second of a similar kind. Apparently there must be some factor or factors somewhere in the organization of the plasma that brings about a total neglect of one stimulus and a complete reaction toward another of a similar nature, since the reaction is not a resultant one. It appears that NOLL's theory alone is insufficient to explain entirely the lack of resultant reaction to two directive forces when applied to an organism as contrasted with the results obtained by physicists in working with inanimate matter. The question, then, of what determines the reaction toward one light and a lack of response toward the second is still unsettled, and the explanation must be deferred to a time when more is known of the intricate mechanism and ultimate organization of the plasma of the cell.

In the experiment with the different incandescent filament lamps, as with the solutions and plates of colored glass used in the earlier work (1), *Pilobolus* fires its sporangia in larger numbers toward the lights in which the proportion of the blue rays is greatest. In other words, it is more responsive to actinic rays. The intensities in the different wave-lengths, as earlier mentioned, are not measurable; but the uniformity of response in favor of the source containing the greater proportion of actinic rays suggests the superiority of the more refrangible rays over the less refrangible rays in causing heliotropic curvatures. This question can be definitely settled only when the intensities of lights of different colors can be measured.

The energy given off by the source of light apparently does not compare in effect with the distribution of the same in different portions of the spectrum. In the experiments using a 20-watt or 16-candle-power tungsten lamp and a 32-candle-power carbon filament lamp the large majority of the sporangia went to the tungsten, although its total energy was but half that of the carbon. From this it is apparent that differences in distribution in the spectrum outweigh in effect the differences in the total energy of the two sources.

The set of experiments using a 16-candle-power tungsten against a tantalum of twice the number of candle-powers showed the discharge to be in favor of the tantalum. At first glance it appears that this contradicts the above results with the carbon and tungsten lamps, and suggests that the total energy of the source does play an important rôle in the results. However, on further consideration, it must be noted that the total number of actinic rays in a tantalum lamp of twice the intensity of the tungsten is probably greater than that in the tungsten. The solution of this point, of course, is bound up with the question of intensity and composition of the sources under discussion and cannot be taken with any degree of finality. With the tungsten lamp of approximately the same intensity as that of the tantalum, the discharge was in favor of the tungsten which emits a larger proportion of the blue rays. The difference as far as distribution in the different wave-lengths of the energy of the tantalum and tungsten lamps is not so great

as is the case of that in the carbon and tungsten lamps. This may account for some of the differences in the distribution of the sporangia in the two cases, when comparing the tungsten with the carbon and with the tantalum.

A comparison of the carbon and tantalum lamps shows again a majority of sporangia discharged on the opening before the tantalum lamp which contained the larger proportion of blue rays.

In the experiments with the rays of different wave-lengths, although the stimuli are both light stimuli, there is a marked difference in composition. We have then an extra factor to deal with; but, as in the experiments with the two nearly equal light sources, there is no sign of a resultant reaction. There is no sign of a change of aim toward one light owing to the presence of a second light. With the tendency of the sporangiophores to discharge toward the blue light, however, it is plain that there is no uniform aim of all the sporangia subjected to the two lights to go to the light having the larger proportion of the blue rays. Why does not *Pilobolus* always discharge toward the source of light having more of the actinic rays? The difference in the length of the light ray does bring a marked variation as regards the numbers fired toward the two sources. Still, some are fired toward the less favorable of the two sources.

The accuracy of aim toward the two lights might well be said to agree in general with that already found for the two light sources used in the above experiments. However, there is a noticeable difference in accuracy of aim toward the different filaments, and that for the most part is in favor of the light with the larger proportion of the more refrangible rays. With the solutions and glass plates used in the earlier work (1) there was a much greater difference noted. There is a probability that the smaller difference may be due to less difference in light intensity, to a smaller difference in composition, and also that there is a limit to the accuracy of response toward any source of stimulation, and that in aiming at the lights in use in these experiments they reached that limit, the less effective lamp being sufficient, or in some cases nearly so, to bring about as accurate a reaction as is possible to the plant.

The question as to what properties of the protoplasm cause it to be more sensitive to rays of one wave-length than to those of another remains unsolved, and, like that as to why it responds to one of two stimuli to the complete exclusion of the other, it must await a better knowledge of the organization of the plasma.

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MORPHOLOGY OF THISMIA AMERICANA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **182**

NORMA E. PFEIFFER

(WITH PLATES VII–XI)

The family Burmanniaceae, chiefly tropical in distribution, is represented by about 50 species in EUBURMANNIAE, 2 in CORSIAE, and 18 in THISMIAE. The geographical range of the first named group is by far the widest; its representatives are found in all tropical regions and extend into the temperate zone. In North America, they are found as far north as Florida, Alabama, and even Virginia. CORSIAE are reported for New Guinea and Chile. THISMIAE are represented by two monotypic genera, *Glaziocharis* and *Triscyphus*, recorded for Brazil, and by 15 species of *Thismia*, if zygomorphic forms are included.

Thismia is subdivided into four groups, *Euthismia*, *Geomitra*, *Bagnisia*, and *Afrothismia*. The *Geomitra* and *Bagnisia* divisions had been described as separate genera by earlier workers, but have recently been included in the genus *Thismia*. To date, the following species in this genus have been described in these regions:

Thismia Brunoniana Griffith (**21**), Tenasserim; T. Gardneriana J. Hooker (**2**), Ceylon; T. macahensis B. & H. (*Ophiomeris macahensis* Miers) (**27**), Rio de Janeiro; T. hyalina B. & H. (*Myostoma hyalina* Miers) (**28**), Organ Mts.; T. Aseroe (*T. Ophiuris*) Beccari (**1**), Borneo and Singapore; T. Neptunis Beccari (**1**), Sarawak; T. javanica J. J. Smith (**9**), Java; T. Winkleri Engler (**7**), Africa; T. crocea Ernst (*Bagnisia crocea* Becc.) (**1**), New Guinea; T. episcopalis F. Muell. (*Geomitra episcopalis* Becc.) (**1**), Borneo; T. clavigera F. Muell. (*Geomitra clavigera* Becc.) (**1**), Sarawak; T. clandestina Miq. (*Sarcosiphon clandestina* Blume) (**12**), Java; T. Rodwayi F. Muell. (**29**), Tasmania; T. Hillii (*Bagnisia Hillii* Cheesem.) (**3**), New Zealand; T. Versteegii J. J. Smith (**12**), Java.

Of these, the first 7 are of the *Euthismia* type, the eighth is the sole representative of *Afrothismia*, and the rest are of the *Bagnisia* or *Geomitra* group. The distribution of these is seen to be practically restricted to the Polynesian Malay region. In view of

this, the finding in the Chicago region of a form closely allied with these last is of decided interest.

Thismia (BAGNISIA) **americana,** nov. sp.—Herbae saprophyticae, tenerae, hyalinae, caulibus simplicibus erectis, radicibus elongatis glabris foliis bracteiformibus. Pedunculi uniflori, erecti vel curvati, 0.3–1 cm. longi. Flores subtiliter virides 0.8–1.5 cm. longi, circiter 6 mm. diametro. Perianthii tubus superus, obovoideo-oblongus, ore constrictus, lobis 6, quorum interiores tres apice conniventes, calyptram 3-stipitatam formantes; lobi alterni equales sed liberi. Stamina 6, fauci affixa, intra tubum deflexa filamentis brevissimis, connectivis maximis membranaceis in tubum deflexum connatis; antherae biloculares, loculis parvis distinctis parallelis, rima longitudinale dehiscentis. Ovarium breve, latum, 1-loculare, placentis 3 parietalibus, in cavo ovarii a pariete solutis. Stylus brevis, crassus, apice trifidus. Ovula numerosa, minuta, anatropa. Fructus turbinato-cupulatus, perianthii circumscisse deciduo truncatus, margine parum elevato cinctus. Semina numerosa, parva, oblonga, albuminosa; testa tenuis, hyalina, reticulata. Embryo parvissimus, in albumine inclusus.

Chicago, Ill., in open prairie, N. E. PFEIFFER.

The plant consists of a white root system, from which arise erect simple floral axes. The roots are about 1 mm. in diameter and vary greatly in length. The flowers are 0.8–1.5 cm. high, borne on an axis 0.3–1.0 cm. high. The perianth tube is conspicuously 6-nerved and with 6 minor nerves. The 3 petals, approximately equal in length to the 3 sepals, are connate at the apex. The mouth of the perianth tube is closed by a disk of tissue, with a central circular aperture surrounded by a raised ring. To this ring the 6 stamens are affixed, and are united into a tube which hangs downward inside the perianth tube. This stamen tube, largely made of the broadened connectives, bears the pollen sacs on the side toward the perianth wall.

The inferior ovary is one-celled, with three placentae which soon become free from the walls, appearing in a central plane as three free columns. The ovules are anatropous, numerous, and

small. There are two integuments. The seed contains a few-celled embryo imbedded in a mass of endosperm.

The entire plant is glabrous and white, save in the 6 divisions of the perianth, where free, and in the disk closing the perianth mouth. Here there is a delicate blue-green color, deeper in the raised ring about the aperture of the disk. Most of the plants have only this colored upper portion above the level of the soil, or of the surrounding moss, etc. The diameter of 5–6 mm. and a height above the soil of 4–6 mm. give an idea of the size of the flower. When the soil is carefully removed, the underground parts are found to be white and semi-transparent; they lie more or less parallel to the surface of the soil, at a depth of a few millimeters. There is no connection with other plants, although the roots of *Thismia* often lie in close juxtaposition with other roots. When the plant is so exposed (figs. 3 and 4), the flower plainly shows the typical THISMIAE structure; a tubular, 6-parted perianth, with the three inner members united at the apex. The leaves, as in other dependent Burmanniaceae, are reduced to white scalelike bracts, so closely appressed to the floral axis that they are readily overlooked.

The material was first discovered in August 1912, in a small space along the margin of a grass field. The habitat may be described as a low prairie, characterized by such plants as *Solidago serotina*, *S. tenuifolia*, *Rudbeckia hirta*, *Eupatorium perfoliatum*, *Asclepias incarnata*, *Iris versicolor*, *Acorus calamus*, and *Agrostis alba vulgata;* and on the soil itself *Selaginella apus*, *Aneura pinguis*, and *Hypnum*. Usually the *Thismia* grows in spots where the soil is not closely covered by *Aneura* and *Selaginella*, but it may be found occasionally among the moss (fig. 3). The little plant is evidently protected both against strong light and great transpiration; but its habitat is in striking contrast to that of most of the other species of *Thismia*, which are found in rich-loamed primeval forests, in regions of great rainfall.

The plants were watched for stages in development during August and the first half of September, to the time when some fruits were obtained. In the season of 1913, visits to the field were made weekly, with the result that flower buds were found on July 1, about a month earlier than the first observation of the previous season.

The indications were that the underground parts had wintered over, although seed-germination may have occurred and been overlooked, since the flower is all that appears above ground.

Earlier descriptions of the Burmanniaceae gave little attention to any but the gross features, which were in the main correctly interpreted. Until recently, the work in anatomy has been done largely by JOHOW (**25, 26**) in *Apteria setacea*, *Gymnosiphon refractus*, *G. trinitatis*, and *Dictyostegia orobanchioides*. The saprophytic forms worked with have scaly rhizomes, from which the flower stalks arise directly, as in *Thismia*. The adventitious roots are reported as being simple, with corky endodermis and a single, greatly reduced vascular bundle of lignified, dotted vessels, arranged in two concentric rings about one central spirally thickened element. The rhizome is described as having a structure much like that of the root. The erect stem or floral axis is credited with having bundles showing distinct xylem and phloem in most forms. The exceptions are *Apteria* and *Gymnosiphon trinitatis*, which have such small bundles that the differentiation is difficult to recognize, according to JOHOW. Nevertheless, he reports all cells of the bundle as lignified.

Recently ERNST and BERNARD (**9–20**) have added largely to the knowledge of the anatomy of different saprophytic forms. They have considered *Thismia javanica* J. J. Sm., *T. clandestina* Miq., *T. Versteegii* J. J. Sm., *Burmannia candida* Engl., *B. Championii*, Thw., and *B. coelestis* Don (*B. javanica* Bl.). In these forms, the vascular elements in the root are much reduced; in *T. javanica* (**10**) the bast alternates with thin-walled parenchyma cells about a central woody area, which is separated from the former by parenchyma cells. The xylem region is figured as consisting of as many as 13 vessels. The fungus occurs in these roots in a patchy arrangement, infecting one group of cortical cells and not another. In the uninfected cells starch is common.

In *T. clandestina* and *T. Versteegii* (**13**), a similar situation as regards arrangement of vascular elements is found, but the xylem is not so conspicuous in amount. In the former species, there is but one subepidermal layer of fungi, in the latter two, the outer of which is the coarser.

The xylem in *Burmannia candida* Engl. (**16**) is represented by but two spirally thickened cells in the central cylinder; occasionally there is only one, seldom three. In addition, there are parenchyma cells and phloem. *B. Championii* (**16**), in contrast to this, has a central cylinder largely made up of xylem elements, with no phloem evident. *B. coelestis* (**19**), a chlorophyll-containing form, shows a similar simple situation as to root anatomy.

In all the forms investigated, the floral axis has well developed collateral bundles. The vascular cylinder is sometimes surrounded by a ring of sclerenchyma tissue, as in *Burmannia candida* and *B. Championii*. The endodermis is usually distinct. Some of the cortical cells contain raphides. In *Burmannia candida* some of the surface cells are much like stomata in form, with pores always open. In *B. coelestis*, a chlorophyllous form, there are normal functioning stomata, in contrast to the usual lack in saprophytic forms.

In *Thismia americana*, superficial examination of the underground structures shows a relatively large number of buds in all stages of development. These occur not only on the main structure, which would appear to be a rhizome, but also on the structures which are very evidently roots appearing at the base of the floral axis. On examination of prepared sections, it appears that the histology of the main structure and of these secondary roots is identical, even to the appearance of a cap at the tip. Because of this fact, these structures, whether primary or secondary, will be referred to as roots. It would appear in field material that roots originally secondary might later appear primary by the dying away of a portion which thus severs the connection with the mother plant.

In the older part of a root of *Thismia*, there is evident a very conspicuous epidermis (figs. 7, 14). This consists of large cells, more or less protuberant, but not developed into hairs in any region. The epidermal cells, in contrast with the cortical cells below the surface, are hyaline. The layer of cells immediately below the epidermis is packed with the thick-walled, branching mycelium of a coarse fungus. In fresh material the mycelium

appears brown. The septate hyphae are usually oriented with the long axis of the root, so that the cross-section of a root shows numerous cut ends (fig. 14), and the longitudinal section a more or less parallel arrangement of the interweaving hyphae (fig. 7). Below this single layer is a region of a varying number of cortical cells containing much finer, thin-walled hyphae. In these deeper parts, masses of protoplasm are frequently evident which strongly suggest the sex organs of some of the Peronosporales. These undoubtedly correspond to the "vesicles" reported by JANSE (**24**) in *Thismia clandestina* (*T. javanica* J. J. Sm.), which he was inclined to believe asexually reproductive bodies. BRUCHMANN considered similar bodies in *Lycopodium annotinum* to be oospores of *Pythium*. In *Thismia* there are also bacteria, probably corresponding to JANSE'S "sporangioles" and "spherules." All these fungal parts are intracellular. A few cells outside of the endodermis are free from fungi. These contain raphides which are common throughout the plant body.

The endodermis consists of a single layer of heavy-walled cells, larger than their neighbors. It encircles a region, probably conducting, of which only a few central cells (3–5) are spirally thickened (figs. 5, 6, 19). They are not lignified, however, and the spiral markings are very fine. Near the point of origin of a floral axis, the number of thickened cells is increased and lignification occurs. These vessels may be seen to connect directly with the vascular elements of the floral axis. The cells adjacent to these spirally thickened vessels do not show the dotted condition that JOHOW reported in the Burmanniaceae considered by him. On the contrary, though they are somewhat elongated, they are nucleate and retain their cytoplasm. They are undoubtedly parenchyma cells, and so the situation is similar to that in *Thismia javanica* J. J. Sm. and other forms investigated by ERNST and BERNARD.

In the outer part of the conducting region are seen a varying number of points of small cells devoid of contents (fig. 6). The arrangement is similar to that reported in other forms by JOHOW and by ERNST and BERNARD. It suggests a radial arrangement, with these groups of cells probably reduced phloem strands without sieve plates.

The growing region resembles that in any root. In a few millimeters at the apex there is a meristematic region of actively dividing cells. Then there is the region of elongation and differentiation. The origin of the different layers would seem to conform to the general situation in monocotyledons, with distinct initials in calyptrogen, dermatogen, plerome, and periblem. This would be in contrast to the situation in *Thismia Versteegii*, where ERNST and BERNARD report a common initial for epidermis and root cap. In *Thismia americana* the tip region of the root is free from fungi, but the tissue formed is rapidly invaded by the mycelium from older parts. In no case was new mycelium seen to penetrate the epidermis and so enter the uninfected region, as reported by JANSE in *Thismia clandestina* (*T. javanica* J. J. Sm.).

Compared with that of the root, the anatomy of the floral axis is complex (figs. 8–10). Here the vascular elements are arranged in a cylinder of 3–6 bundles. In the early stages the number is very apt to be 3; in the older axis, near the apex, there are divisions of the original bundles, giving a larger number, frequently 6. More may be seen where branches go from the original bundles.

Each bundle consists of definite xylem elements and a mass of cells with slightly thickened walls (fig. 10). The xylem is slower to appear than the latter, which are early clearly distinguishable (fig. 8). In mature parts, the small clear cells appear in the same relation to xylem as phloem usually does, but they show no sieve plates so far as can be determined. Nevertheless, it seems probable that these elongated cells function as phloem. The xylem vessels have lignified and spirally thickened walls and their number varies from 2 to 15 in each bundle. The large number, the conspicuous spiral thickenings, and the lignification, as well as the greater and more definite phloem development, are quite in contrast to the condition found in the root.

A single strand of a few xylem vessels and phloem cells supplies each of the bractlike leaves (fig. 11), which are very thin and relatively broad. There are also branches of the main cylinder supplying the floral parts and producing the conspicuous nervation of the perianth.

There is no definite endodermis or pericycle in the floral axis,

nor is there a ring of sclerenchyma about the vascular cylinder as reported for some other burmanniaceous forms. As might be expected, no stomata were found; the corollary of no air spaces follows logically.

The floral axes appear to arise a short distance back of the tip of the root (figs. 4, 20). The first external evidence of their development is a single small excrescence (fig. 20*a*). Later two growing points, usually point upward, are distinguishable. Growth is quite rapid and soon results in a first root and a floral axis (fig. 20), from the base of which other roots take origin (figs. 21, 22).

In prepared material, the earliest stage in the development of the bud is shown in fig. 12. A region of rapidly dividing cells occurs below the epidermis in a somewhat arched mass. At this stage the main root itself is in such an undifferentiated condition that the endodermis and neighboring tissues are not yet distinct. In slightly older stages, a break is seen to occur between the cortical cells of the root and the growing cells of the endogenous branch, which now has a slightly lobed margin (fig. 13). At this time, the beginning of the first root to be developed may be seen (figs. 13, 19) as a mass of meristematic tissue to one side of the floral bud. By rapid growth the root overtakes the stem from which it originated, so that when the two structures have emerged a little beyond the boundary of the main root, they are about the same size (figs. 14, 15). At this time the floral part is still protected by the arch of primary root cortical tissue, but the root tip soon breaks through the cortex, becoming much the longer organ (fig. 16). A renewed growth of the floral axis and the development of other secondary roots from the main floral axis (fig. 17) finally result in a horizontal position of the first root, which had previously stood erect beside the floral axis.

In the development of the axis, the rudiments of the bract leaves appear laterally (fig. 17). After elongation and the development of the leaves, the differentiation of the floral parts occurs (fig. 18). The perianth tube develops early. At the same time, the stamens begin their development. The ovary with its ovules is late in appearance, a case similar to that in Orchidaceae. When

the ovules are first beginning to be evident, the microsporangial tissue is well defined.

There are, as usual, four microsporangia in early stages (fig. 23), which later fuse to form the two pollen sacs (fig. 28). The early stages show the stamens bent inward and downward, but not connected with each other. Later growth, particularly of the connectives, makes a continuous tube, which is, however, easily separated into the constituent stamens (fig. 28). This tube extends beyond the sporangia, which occur on the side toward the perianth wall. In mature stages the pollen grains are slightly oval. They are very pale, almost transparent, with a pale green cast, due to little bodies, probably fat, many of which also occur in the perianth parts. The microspores are loose and free, not massed together as in pollinia of orchids. Before shedding, the generative nucleus divides (fig. 29). A granular mass in the spore suggests the presence of a prothallial cell, but failure to secure stages in the present investigation must leave this doubtful at present.

The ovules, very many in number, develop on parietal placentae (fig. 24), which swing free from the ovary walls in the center (fig. 18). At this level ovules project on all sides of the placental column. In early stages the numerous primordia appear as in fig. 18. The fully developed ovule shows two integuments (figs. 26–27). It is anatropous, with a long funiculus. As JOHOW (**26**) and TREUB (**30**), and lately ERNST and BERNARD, reported, there is a conspicuous differentiation of a few of the nucellar cells at the base of the embryo sac. This seems to have no significance at present.

The seed is minute, with a testa two cells in thickness. The outer layer is composed of large, almost transparent cells. The inner one is constructed of smaller cells, with more contents, often appearing oil-like. The seed has a very evident endosperm, with cells of relatively large diameter, and an inconspicuous embryo of a few cells (fig. 30). In all respects it seems to agree with the accounts of TREUB (**30**) and JOHOW (**25, 26**), the former of whom first correctly interpreted the endosperm. MIERS, in 1866, declared that the seed of *Myostoma* contained no embryo. Later, GRIFFITH interpreted the entire content of the seed as embryo. TREUB,

in 1883, found a weakly developed embryo of 3 or 4 cells in a mass of endosperm in *Burmannia maburnia* and *B. javanica*. JOHOW, in 1885, reported a similar situation in *B. capitata* with a 10-celled embryo, and in *Apteria* one with 4 cells. In *Thismia javanica*, ERNST and BERNARD (**11**) have found a more strongly developed embryo with 4–6 tiers of cells. *Thismia clandestina* (**14**) has a still better differentiated embryo, with a 3-celled suspensor above a spherical body, the outer cells of which are differentiated from the inner. *T. Versteegii* (**14**), a closely related form, has on the other hand a simple embryo. *Thismia americana* then would seem, in its embryo situation, to resemble this last species and forms like *Burmannia javanica* and *B. maburnia*.

No case of polyembryony has been found, such as was reported by ERNST (**8, 20**) in *Burmannia coelestis* Don, a form developing embryos apogamously. Here the number of embryos was one, two, or three, dependent on whether the egg alone functioned, or the synergids were also active. In the earlier history ERNST found no reduction division to occur in the formation of "megaspores." Stages have not yet been obtained in *Thismia americana* to work out the sequence here, but assuredly, at maturity, only one embryo in each seed has so far been found.

The arrangement of parts in the flower seems such that insect pollination would be necessary, unless a situation similar to that in *Burmannia candida* Engl. and *B. Championii* existed. Here the pollen grains germinate in the sporangia, and the pollen tubes grow toward the style branches. No indication of this condition was found in *Thismia americana*.

Up to date, the few attempts at germinating the tiny seeds have been fruitless. It is to be hoped that a larger harvest may give a better opportunity for positive results. The relation of the fungus inhabitants to the developing plant might be better worked in this connection than with the mature plant. Since the fungi occur in the root, the absorptive region, and not in the stem, they would seem to have some connection with water and food supply. Microchemical tests show that in the root there is a very large supply of reserve food in the form of oils or fats. Contrary to the results of ERNST and JOHOW, no sugars or starch are present in

large enough amounts to detect by microchemical means. As indicated above, large amounts of calcium oxalate are present in the form of raphides throughout the parenchyma tissues of the plant body. This is probably to be related to the presence of the fungi.

The green oil-like bodies in the perianth parts gave in spectroscopic tests an absorption in the blue band. The coloring matter is not easily soluble in alcohol.

Further features in the morphology and cytology of this plant will be presented in a later paper.

Summary

1. The characters of *Thismia americana* are deemed sufficiently different from those of other members of the genus to warrant the description of a new species.

2. The main subterranean structure cannot be distinguished from a root, having a similar anatomical structure, including a root cap.

3. The root shows great reduction. The xylem is represented by 3–5 central spiral elements, the phloem by 4–6 small groups of cells. A radial arrangement is suggested in the grouping.

4. The vascular cylinder of the floral axis consists of 3–6 bundles, with xylem and phloem collaterally arranged. The xylem is composed of spiral lignified vessels. No sieve plates are distinguishable in the phloem.

5. The floral axis and first root arise from the main root endogenously. Other secondary roots arise from the base of the floral axis bud.

6. The ovary is slower in development than the other floral parts. Microsporangia are well developed when ovules first appear.

7. The ovules are anatropous, and have two integuments.

8. The embryo, consisting of a few cells, is imbedded in a mass of large-celled endosperm.

The author wishes to express her thanks to Professor JOHN M. COULTER, Dr. CHARLES J. CHAMBERLAIN, and Dr. W. J. G. LAND, under whose direction this investigation was carried on.

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EXPLANATION OF PLATES VII–XI

All figures, except 1, 2, 3, 4, 20, 21, 22, and 28, were drawn at the level of the table with the aid of a Spencer camera lucida under Spencer objectives 16, 4, or 1.8 mm., and oculars 2, 4, or 8. The following abbreviations are used: *b*, bud; *br.* bract; *e*, epidermis; *fa*, floral axis; *ii*, inner integument; *oi*, outer integument; *lt*, leaf trace; *l*, fungus infected layers; *m*, microsporangia; *mr*, main root; *o*, ovary; *p*, phloem; *p'*, perianth; *pw*, perianth wall; *rc*, root cap; *s*, style; *sr*, secondary root; *s'r'*, second secondary root; *x*, xylem.

FIG. 1.—Side view of plant of *Thismia americana*, in situ; ×2.

FIG. 2.—View of flower from above; petals cut apart at apex and folded back; ×4.5.

FIG. 3.—View from above of group of flowers undisturbed in natural situation; the oldest flower appears at the right; ×4.5.

FIG. 4.—View from above of plants from which the soil has been removed; the white root portions are evident with their buds; ×2.6.

FIG. 5.—Longitudinal section of central cylinder of root; ×266.

FIG. 6.—Cross-section of central cylinder of root; ×266.

FIG. 7.—Portion of root in longitudinal section, showing subepidermal fungus infected layers, and epidermis free from fungi; ×266.

FIG. 8.—Cross-section of central region of young erect axis, showing early phloem development; ×266.

FIG. 9.—Diagrammatic cross-section of erect axis and bract leaves; the outlined portion of the axis proper is shown in fig. 10, of the leaf in fig. 11, with higher magnification; ×35.

FIG. 10.—Detailed drawing of portion of cross-section of floral axis; one leaf trace supplies the next leaf; ×266.

FIG. 11.—Detail of portion of cross-section of leaf, showing single, simple bundle; ×266.

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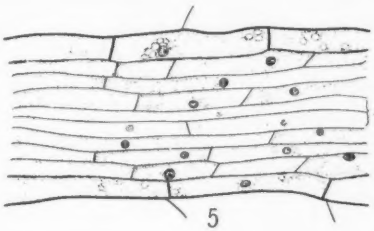

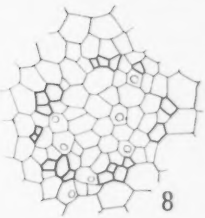

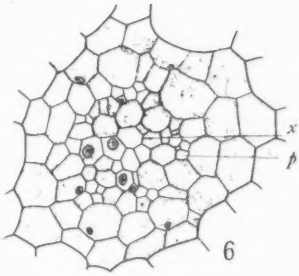

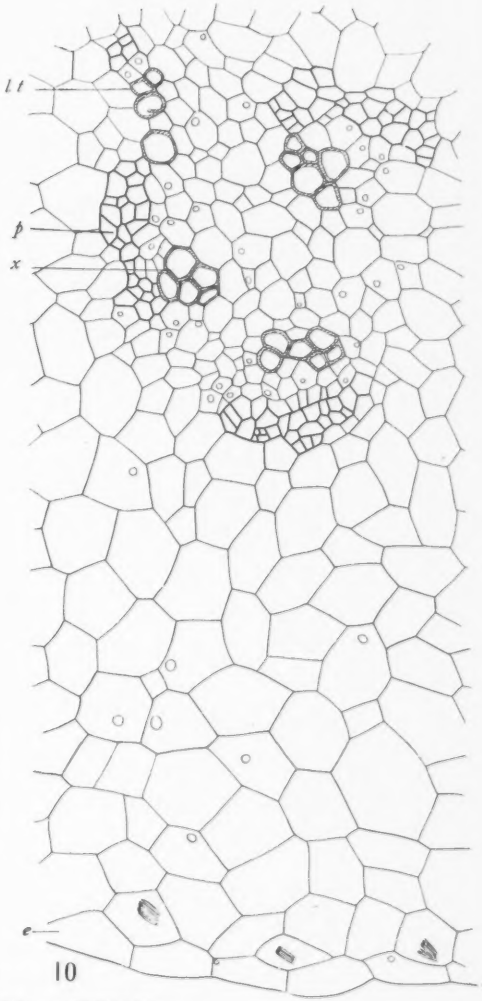

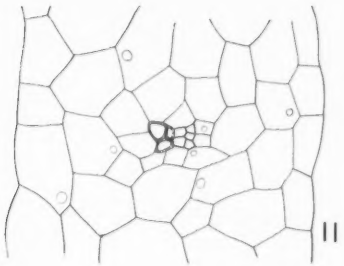

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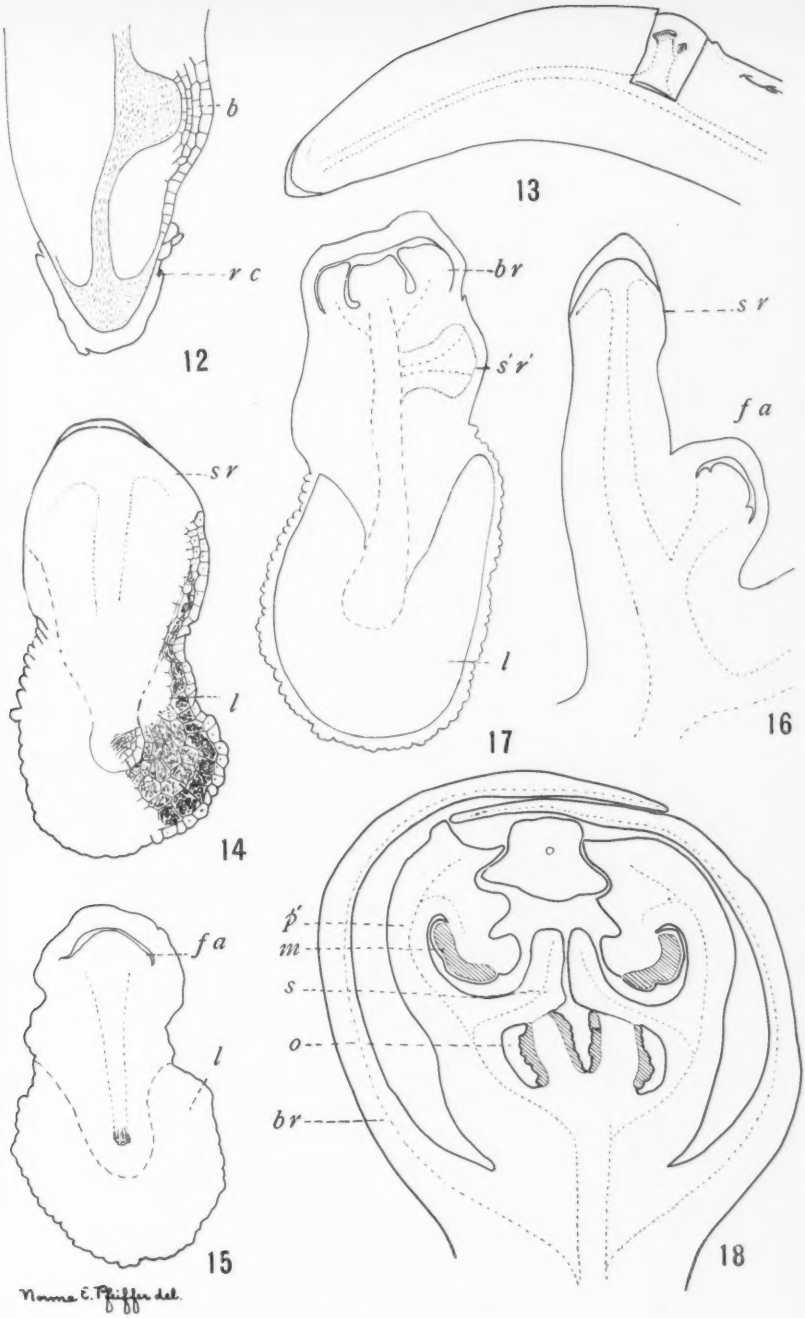

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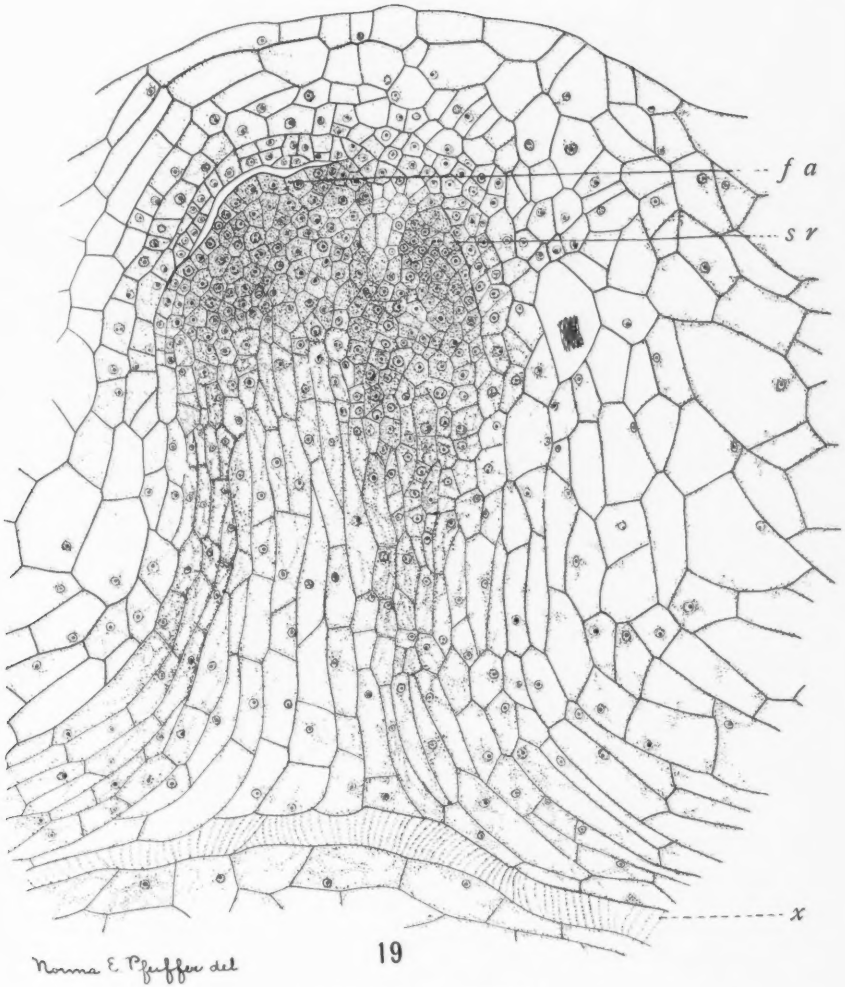

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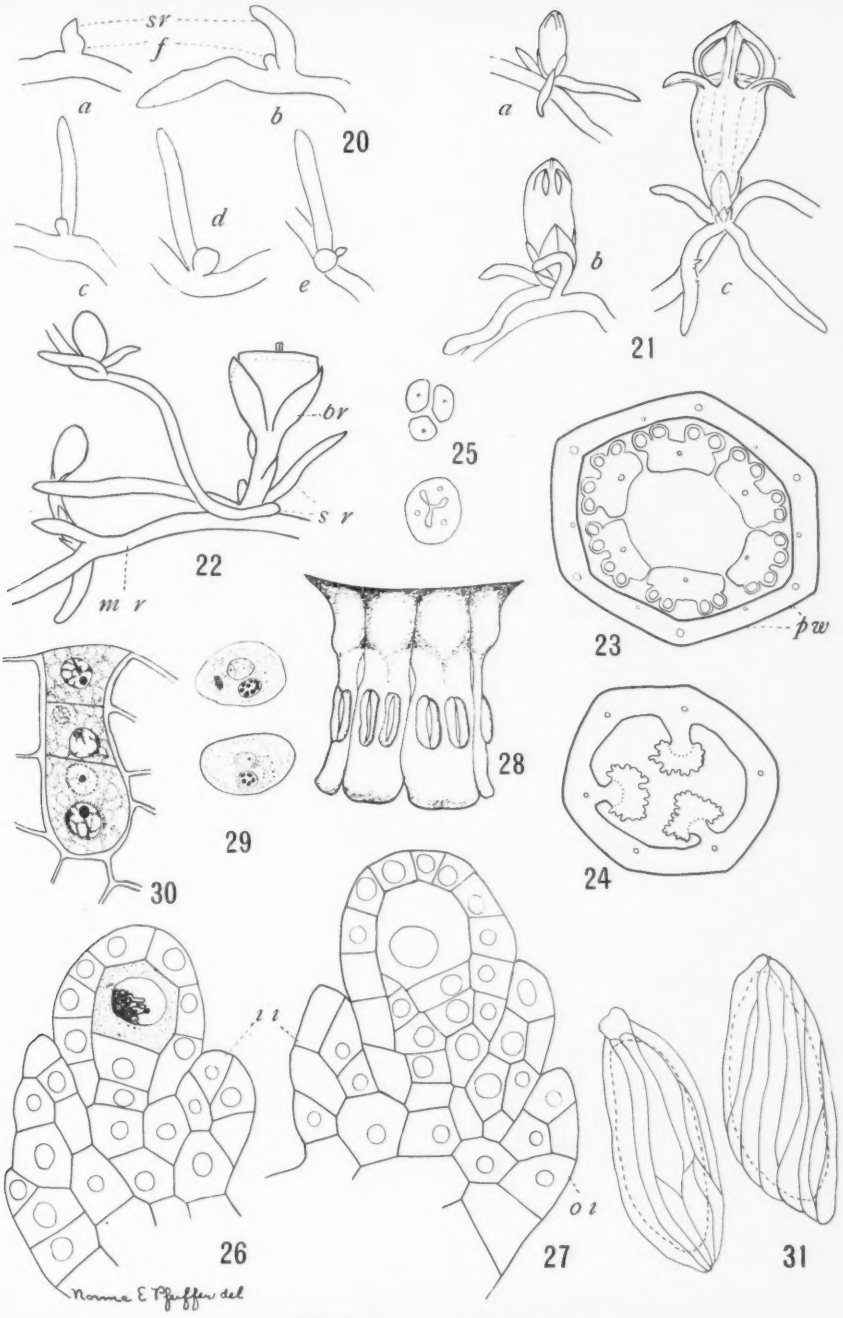

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FIG. 12.—Longitudinal section of tip of root, with young bud just developing; its proximity to the root cap is striking; ×52.

FIG. 13.—Older bud, in which the floral axis and secondary root tip have begun to differentiate; outlined portion in detail in fig. 19; ×26.

FIG. 14.—Cross-section of main root with secondary root; ×52.

FIG. 15.—Same main root, showing floral axis in neighboring section; ×52.

FIG. 16.—Later stage, where secondary root has elongated more rapidly than floral axis; longitudinal section of main root; ×52.

FIG 17.—Cross-section of main root, longitudinal of floral axis, at base of which second secondary root is developing; ×52.

FIG. 18.—Young flower in longitudinal section, showing arrangement of parts; ×52.

FIG. 19.—Detailed drawing (see fig. 13) to show relation of rudiments in bud; ×275.

FIG. 20.—Early stages in development of root and floral axis, as seen in habit material; *e*, same plant as *d* seen from above; ×4.

FIG. 21.—Subsequent stages; ×2.

FIG. 22.—Later stage, showing fruit; other floral axes arising on primary root and on secondary root.

FIG. 23.—Cross-section of young flower, showing stamens; ×26.

FIG. 24.—Cross-section of same flower through ovary; ×26.

FIG. 25.—Cross-section of style of same flower; ×26.

FIGS. 26, 27.—Ovules at megaspore mother cell stage; ×835.

FIG. 28.—Stamen tube removed from flower, as seen from side toward perianth wall; the pollen sacs have dehisced longitudinally; ×8.

FIG. 29.—Microspores before shedding; the generative cell about to divide; remains of a prothallial cell(?) in one; ×835.

FIG. 30.—Embryo imbedded in endosperm; ×835.

FIG. 31.—Seeds at maturity; ×87.

CONCERNING THE PRESENCE OF DIASTASE IN CERTAIN RED ALGAE

E. T. Bartholomew

Introduction

In most starch-forming plants the starch grains are deposited in the plastids. The work of both early and recent investigators shows, however, that this is probably never true for the red algae. In this group of plants the grains are described as being formed in the cytoplasm, outside the plastids and often apparently quite independent of them.

In the red algae the grains, which resemble the starch grains of higher plants, do not usually give the customary blue color when treated with iodine or zinc chloriodide. In a few species, for example *Lorencia* sp., *Polysiphonia nigrescens*, and *Ceramium tenuissimum*,[1] the color with iodine varies from violet to almost blue, but in most species it ranges from light brown to wine red. There is such a wide divergence of reaction in the different forms tested that Oltmanns[2] suggests the possibility that each species will be found to give its own characteristic color reaction.

Saiki[3] found that by applying strong solutions of malt extract to commercial food preparations made from various red algae, such as *Gelidium* sp., *Porphyra* sp., and *Chondrus crispus*, no digestion whatever of the carbohydrates took place. From this and other experiments he concludes that the polysaccharid carbohydrates in these algal food preparations are not readily transformed into sugar by carbohydrate-digesting enzymes of animal origin, and scarcely more so by vegetable enzymes or bacteria.

König and Bettels[4] hydrolyzed, with acids, the carbohydrates in *Porphyra* sp., *Gelidium* sp., and commercial compounds com-

[1] Oltmanns, F., Morphologie und Biologie der Algen **2**:148.

[2] *Ibid.*

[3] Saiki, T., The digestibility and utilization of some polysaccharid carbohydrates derived from the lichens and red algae. Jour. Biol. Chem. **2**:251–265. 1906.

[4] König, J., and Bettels, J., Die Kohlenhydrate der Meeresalgen und daraus hergestellter Erzeugnisse. Zeitschr. Untersuch. Nahrungs- und Genussmittel **10**: 457. 1905.

posed of red algae. Their results showed the presence of galactose, fructose, and glucose in varying quantities. In carrageen (*Chondrus crispus*), MÜTHER and TOLLENS[5] found galactose and probably other hexoses belonging to the fructose and glucose groups.

The grains in the red algae often show the physical characteristics of the common starch grains of green plants as to hilum, striation, and effect of polarized light, but their place of deposition, their behavior toward iodine and zinc chloriodide, their sugar extracts, and their apparent resistance to the action of malt extract would tend to prove that they are not true starch. The grains appear and disappear at various times during the life of the plant, thus suggesting the presence of an enzyme which is an active agent in bringing about their decomposition.

The following experiments were performed that further evidence might be gained as to the nature of the substance composing the starchlike grains in the red algae, by determining whether or not the diastase that acts on the grains in these plants will also digest the common starch isolated from the higher plants. So far as is known to the writer, this is the first attempt in this direction, and a preliminary statement of the results is herewith recorded.

Material and methods

1. SPECIES FROM WHICH EXTRACTS WERE MADE

Polysiphonia variegata Ag., *Dasya elegans* Ag., *Agardhiella tenera* (J. Ag.) Schmitz, and *Ceramium*[6] lent themselves most readily to experimentation because of their abundance and the ease with which they could be obtained. *Grinnellia americana* Harv., *Griffithsia globifera* J. Ag., and *Chondrus crispus* (L.) Stack. were also used and tended to show the same results as those obtained from the foregoing species, but sufficient quantities of the latter named plants could not be procured at the time of these experiments to warrant the drawing of definite conclusions.

[5] MÜTHER, A., and TOLLENS, B., Ber. Deutsch. Chem. Gesells. **37**: 298–305, 306–311. 1904.

[6] Several species were used in the same mass of material.

2. METHOD OF EXTRACTION AND AMOUNTS OF FINAL PRECIPITATES

The extracts were made according to modified Buchner and Litner methods. Fresh vigorous algae were taken from the sea water and quickly rinsed under the tap. The excess tap water was removed by filter paper or cheese cloth. The algal material was then immersed in 95 per cent alcohol for 20 minutes. Having been freed from excess alcohol, it was next plunged into acetone for 10 minutes. The excess of this solution having been removed, the material was placed in fresh acetone for two minutes, partially dried by suction, and placed in an oven at 35°–40° C. until dry. Upon being taken from the oven, the dried material was cooled in a desiccator, thoroughly pulverized, placed in three times its amount of 20 per cent alcohol, and left standing 18–24 hours. At the end of this period the substance was filtered by suction through a Buchner funnel lined with cheese cloth and filter paper (it was necessary to filter the material by suction because of its viscosity), and to the liquid thus drawn off was added two and one-half times its volume of 95 per cent alcohol. After being filtered as above, the precipitate was washed in equal parts of ether and absolute alcohol, dried in a desiccator, and after being pulverized the extract was ready for use. The extraction treatment did not remove all of the color, so that the precipitates varied from brownish to light red.

Because of the difficulty experienced in filtering, the final precipitates were probably far from pure, but the following figures will show the amounts of precipitates obtained from the different species:

Polysiphonia variegata	21.00	grams	powder*	—0.15	gram	final ppt.
Ceramium	51.00	"	"	—0.15	"	" "
Dasya elegans	53.03	"	"	—0.06	"	" "
Agardhiella tenera	53.465	"	"	—0.23	"	" "
Agardhiella tenera	17.00	"	"	—0.106	"	" "

*Obtained after material had been dehydrated, desiccated, and pulverized.

3. ALGAL EXTRACTS AND COMMERCIAL DIASTASE

Solutions of various concentrations were made from the algal extracts, but the one giving the best results consisted of 0.03 gm. of extract dissolved in 5 cc. of distilled water. Owing to the

nature of the extracts, solutions of this concentration were quite viscous. In most cases the viscosity would about equal that of a 75 per cent solution of glycerine and water. That a check might be had as to the time required for action and as to the final results, solutions of commercial diastase were also used. The solutions were of the same concentration as those of the algal extracts and were applied and tested in the same manner in every case. Both Eimer and Amend's and Merck's diastase were used, with no apparent difference in the results.

4. STARCH PASTE

Corn starch was used in all experiments. The paste used was made by adding 0.25 gm. of starch to 100 cc. of water and boiling 6 minutes. The boiling, though done slowly and in an Erlenmeyer flask plugged with cotton, probably caused 10 per cent of the water to be vaporized. No water was added to make up for this loss. New solutions of paste were made for each series of experiments.

5. CONTROL MEASURES

To determine as far as possible all sources of error, the following control measures were taken:

a) The "A, B, and C" parts of Fehling's solution were mixed just preceding each series of experiments.

b) Tubes of untreated paste were tested each time and in the same manner as those tubes containing the paste plus the extracts.

c) To detect any error due to the presence of an active agent in the distilled water, tubes of the paste were treated each time with distilled water only and tested in the regular manner.

d) Toluol was used as a sterilizing medium, except in the Van Tieghem tests, in which 10 per cent alcohol was used.

e) All containers and instruments were carefully sterilized with a strong solution of potassium bichromate and sulphuric acid.

6. TEST METHODS

A. *Digestion in test tubes*

The proportionate amounts of starch, algal extracts, commercial diastase, and distilled water used in each series of tests were as follows:

Test tube no. 1–5 cc. starch paste plus 1 cc. algal extract
" " " 2–5 cc. " " " 1 cc. " "
" " " 3–5 cc. " " " 1 cc. commercial diastase
" " " 4–5 cc. " " " 2 cc. " "
" " " 5–5 cc. " " " 1 cc. distilled water
" " " 6–5 cc. " " " 2 cc. " "
" " " 7–5 cc. untreated starch paste
" " " 8–2 cc. algal extract solution
" " " 9–2 cc. commercial diastase solution

When the experiments were to run for a longer period, the amounts were increased, but the same proportions were maintained. At the end of certain periods, indicated in the tables, 2 cc. of the solution were taken from tube number one and placed in two smaller tubes, 1 cc. in each tube. This process was repeated until test samples had been taken from each of the 7 tubes. One of each pair of small tubes was treated with iodine and the other with Fehling's solution. The experiments were all kept at room temperature, which was rather low, the daily average being 18°.6 C.

B. *Digestion in Van Tieghem cells*

To determine microscopically the effect of the algal extract upon unboiled starch, small quantities of dry corn starch were placed in four Van Tieghem cells. Three of these were treated with extracts from *Polysiphonia*, *Agardhiella*, and *Ceramium*, the fourth with distilled water to act as a control.

7. TERMS USED IN TABLES TO DESIGNATE COLOR REACTIONS AND AMOUNTS OF PRECIPITATES

That the standard for the designation of iodine color reactions and for the amounts of copper precipitate might be as nearly as possible the same for each series of experiments, two sets of terms were adopted. These may be interpreted in the following manner by referring to PRANG'S *Standard of colors* (1898):

IODINE COLOR REACTIONS

As specified in tables	As illustrated by chart
1. Blue	Plate I, 1–B
2. Tinge of purple	" I, 1–BV
3. Blue-purple	" II, 1–BV
4. Purple	" II, 1–V
5. Light purple	" II, 2–V
6. Very light purple	" II, 3–V
7. Purple-brown	" V, 5–O
8. Brown	" III, 3–OYO

*Fehling's solution precipitates**

As specified in tables	Algal extract	Commercial diastase
1. Trace	Plate II, 6–OYO	Plate II, 6–YYG
2. Very slight	" II, 5–O	" II, 4–Y
3. Slight	" I, 6–OYO	" II, 2–Y
4. Small	" I, 5–O	" I, 4–Y
5. Fair	" I, 4–ORO	" I, 3–Y
6. Medium	" I, 3–ORO	" I, 2–Y
7. Marked	" I, 3–RO	" I, 1–Y
8. Very marked	" I, 2–RO	" I, Y

*Although the precipitates from the two different extracts were of different colors, the terms used designate, as nearly as could be estimated, equal amounts of the two precipitates.

These designations were of course only relative, but they served very well as a guide. The colors designated in the tables were recorded within a few minutes after the application of the testing reagents.

Results

1. DIGESTION IN TEST TUBES

Tables I–IV were chosen promiscuously from a large number of tables, and are used as types to show the effect of the algal extract on starch paste and its rate of action in comparison with that of commercial diastase.

TABLE I

EXTRACT FROM CERAMIUM

Amounts of various solutions	After 11 hours		After 24 hours	
	Iodine color reactions	Amts. ppt. with Fehling's solution	Iodine color reactions	Amts. ppt. with Fehling's solution
1 cc. algal extract+5 cc. starch paste	Tinge of purple	Trace	Blue-purple	Slight
2 cc. algal extract+5 cc. starch paste	Blue-purple	Slight	Purple	Small
1 cc. commercial diastase+5 cc. starch paste	Light purple	Fair	Light purple	Medium
2 cc. commercial diastase+5 cc. starch paste	Very light purple	Fair	Light brown	Marked
1 cc. distilled water+5 cc. starch paste	Blue	None	Blue	None
2 cc. distilled water+5 cc. starch paste	Blue	None	Blue	None
Control, 5 cc. starch paste	Blue	None	Blue	None
2 cc. algal extract	.....	None	.....	None
2 cc. commercial diastase	.....	Medium	.....	Medium

The action of the algal extract is very slow when compared with that of commercial diastase, as is shown in the preceding tables I–IV. To determine whether the action of the algal extract would

TABLE II

EXTRACTS FROM POLYSIPHONIA VARIEGATA

AMOUNTS OF VARIOUS SOLUTIONS	AFTER 12 HOURS		AFTER 36 HOURS	
	Iodine color reactions	Amts. ppt. with Fehling's solution	Iodine color reactions	Amts. ppt. with Fehling's solution
1 cc. algal extract+5 cc. starch paste	Tinge of purple	Slight	Light purple	Medium
2 cc. algal extract+5 cc. starch paste	Blue-purple	Small	Very light purple	Medium
1 cc. commercial diastase+5 cc. starch paste	Purple-brown	Marked	Brown	Very marked
2 cc. commercial diastase+5 cc. starch paste	Brown	Marked	Brown	Very marked
1 cc. distilled water+5 cc. starch paste	Blue	None	Blue	None
2 cc. distilled water+5 cc. starch paste	Blue	None	Blue	None
Control, 5 cc. starch paste	Blue	None	Blue	None
2 cc. algal extract	.....	None	.....	None
2 cc. commercial diastase	.....	Medium	.....	Medium

TABLE III

EXTRACTS FROM AGARDHIELLA TENERA

AMOUNTS OF VARIOUS SOLUTIONS	AFTER 16 HOURS		AFTER 28 HOURS	
	Iodine color reactions	Amts. ppt. with Fehling's solution	Iodine color reactions	Amts. ppt. with Fehling's solution
1 cc. algal extract+5 cc. starch paste	Blue	None	Tinge of purple	Trace
2 cc. algal extract+5 cc. starch paste	Tinge of purple	Trace	Blue-purple	Slight
1 cc. commercial diastase+5 cc. starch paste	Purple-brown	Medium	Brown	Very marked
2 cc. commercial diastase+5 cc. starch paste	Brown	Medium	Brown	Very marked
1 cc. distilled water+5 cc. starch paste	Blue	None	Blue	None
2 cc. distilled water+5 cc. starch paste	Blue	None	Blue	None
Control, 5 cc. starch paste	Blue	None	Blue	None
2 cc. algal extract	.....	None	.....	None
2 cc. commercial diastase	.....	Medium	.....	Medium

cease before digestion was complete, experiments were set up as before but allowed to run for a longer time. The results are shown in table V.

TABLE IV

EXTRACT FROM DASYA ELEGANS

AMOUNTS OF VARIOUS SOLUTIONS	AFTER 17 HOURS		AFTER 25 HOURS	
	Iodine color reactions	Amts. ppt. with Fehling's solution	Iodine color reactions	Amts. ppt. with Fehling's solution
1 cc. algal extract+5 cc. starch paste	Blue	None	Blue ?	Trace
2 cc. algal extract+5 cc. starch paste	Blue ?	Trace	Tinge of purple	Very slight
1 cc. commerical diastase+5 cc. starch paste	Very light purple	Marked	Brown	Very marked
2 cc. commercial diastase+5 cc. starch paste	Purple-brown	Marked	Brown	Very marked
1 cc. distilled water+5 cc. starch paste	Blue	None	Blue	None
2 cc. distilled water+5 cc. starch paste	Blue	None	Blue	None
Control, 5 cc. starch paste	Blue	None	Blue	None
2 cc. algal extract	.....	None	.....	None
2 cc. commerical diastase	.....	Medium	.....	Medium

TABLE V

EFFECTS OF LONGER CONTINUED ACTION OF THE ALGAL EXTRACTS AND THEIR COMPARATIVE RATES OF ACTION

AMOUNTS OF SOLUTION ON 5 CC. OF STARCH PASTE	AFTER 3 DAYS		AFTER 6 DAYS	
	Iodine color reactions	Amts. ppt. with Fehling's solution	Iodine color reactions	Amts. ppt. with Fehling's solution
2 cc. Agardhiella extract	Purple	Fair	Very light purple	Very marked
3 cc. Agardhiella extract	Light purple	Medium	Very light purple	Very marked
2 cc. Ceramium* extract	Tinge of purple	Very slight	Blue-purple	Slight
3 cc. Ceramium* extract	Blue-purple	Slight	Purple	Fair
2 cc. Polysiphonia extract	Blue-purple	Medium	Very light purple	Very marked
3 cc. Polysiphonia extract	Purple	Marked	Very light purple	Very marked
2 cc. distilled water	Blue	None	Blue	None
3 cc. distilled water	Blue	None	Blue	None
Control, 10 cc. starch paste	Blue	None	Blue	None

*It took nine days for *Ceramium* to complete digestion.

2. DIGESTION IN VAN TIEGHEM CELLS

The cells were examined daily and it was found that in those containing the starch treated with the algal extracts, very noticeable corrosion of the grains was taking place. The rates of digestion agreed very well with the tabulated results in table V, *Polysiphonia* and *Agardhiella* acting more rapidly than *Ceramium*. The results thus obtained in the first trial were confirmed by repeating the experiment several times. Marked acceleration of corrosion was obtained by placing the cells in a warming oven at a temperature of 26° C. No corrosion could be detected in the control cells.

Discussion

That the disappearance of the starchlike grains in the living tissues of the red algae is due to the presence of some catalytic agent, which may be extracted and applied to corn starch and its derivatives with positive effects, is demonstrated by the results recorded in the foregoing tables. The action of this extract when applied to starch paste is very similar to that of commercial diastase. This is shown by the fact that in both cases the digestion of the starch appeared to be a series of processes leading more or less gradually from the amyloses down through the dextrins until amylolytic action was complete. This is brought out clearly not only by variations in color, but also by the fact that often when the iodine would indicate a marked degree of change in the solution, still the Fehling's test would show the presence of but very little sugar. It would appear from this that the algal extract, like the diastase of the higher plants, is not one simple enzyme but a complex of amylases and dextrinases. Then, if we may argue from analogy, the grains in the red algae, commonly referred to as starch, are probably, as MEYER[7] and others suggest, a combination of both starch and dextrins, or, as BÜTSCHLI[8] thinks, possibly a transitional stage between amyloporphyrin and amyloerythrin.

No direct evidence was obtained by experimentation as to whether or not this carbohydrate-digesting enzyme of the red algae

[7] MEYER, A., Untersuchungen über die Stärkekörner. Jena. 1895.

[8] BÜTSCHLI, O., Notiz über d. sogen. Florideen Stärke. Verh. Naturf. Med. Ver. Heidelberg N.F. **7**:519–528. 1903.

might also act as a synthetic agent in forming the grains in question. BELZUNG[9] found that in some species the younger the grains the more nearly they came to giving the normal blue color reaction when treated with iodine. Hence, if this enzyme does act synthetically, it appears that, in some cases at least, the action is just the reverse of that which takes place in some of the higher plants, for example, in *Pisum sativum*, in which the erythrodextrins are formed first, the amylases not appearing until the grains are almost mature.

The difference in rate of amylolytic action between the algal extract and the commercial diastase is very noticeable. This is already indicated in tables I–IV. This variation may have been due to difference in concentration. The algal extracts were known to be far from pure, and it was for this reason that solutions of rather high concentration were applied to the starch paste. Table V indicates that there is also considerable dissimilarity in digestive power between the algal extracts themselves. Here again is the possibility that this may be attributed to an inequality in purity, for the extracts did not all present the same degree of difficulty in filtration. However, the explanation is hardly to be looked for in this direction, for although *Agardhiella* and *Polysiphonia* extracts were more difficult to filter than was the *Ceramium* extract, yet the two former completed amylolysis in 6 days, while the latter required 9 days under the same conditions, as is shown in table V.

As a result of recent experiments, ACHALME and BRESSON[10] maintain that diffusion is an important factor in the rate of enzymatic action. They found that an increase in viscosity of the solution meant a like decrease in the rate of action of invertase upon saccharose. They also maintain that this is true for emulsin, trypsin, and for organic oxidases. As has been said, the viscosity of the algal extracts would about equal a 75 per cent solution of glycerine in water. That this condition influenced the amylolytic action

[9] BELZUNG, E., Recherches morphologiques et physiologiques sur l'amidon et les grains de chlorophylle. Ann. Sci. Nat. Bot. VII. **5**:223–228. 1887.

[10] ALCHALME, P., and BRESSON, M., Influence de la viscosité du milieu sur les actions-diastasiques. Compt. Rend. **152**:1328–1330. 1911; also Du rôle de la viscosité dans les variations de l'action de l'invertine suivant les concentrations en saccharose. Compt. Rend. **152**:1420–1422. 1911.

of the extracts is not evident. It is true that the viscosity of the algal extracts greatly exceeded that of the commercial diastase and that action was very much more rapid in the latter than in the former; but this may have been due to some other factor, for although the extract from *Polysiphonia* was quite noticeably more viscous than that from *Ceramium*, yet the former completed digestion in two-thirds of the time required by the latter. Here, as before, we cannot draw definite conclusion, for there may have been a marked variation in purity between the two extracts. In some of the higher plants we find diastases that work very slowly, and it is not impossible that the algal diastases are also of this nature. It is a well known fact that by the addition of very small amounts of such substances as free mineral acids, neutral phosphoric acid compounds, salts of aluminium, and asparagin salts, the amylolytic action of the diastase from higher plants is very much accelerated, and it might be mentioned here that small amounts of aluminium acetate and sodium chloride had the same accelerating effect upon the algal extracts.

The Van Tieghem tests were of interest not only because they corroborated the macrochemical tests, but also because they showed that the manner of attack of the algal extracts was such that the starch grains were corroded. This would indicate that at least the most active enzyme present was a translocation rather than a secretion diastase.

The contrast between the colors of the precipitates of the different mediums tested with Fehling's solution was very marked. The precipitates in the tubes containing the paste treated with algal extract was of the usual "brick-red" color. Paste treated with commercial diastase gave a light yellow precipitate, even though the tubes were allowed to stand for a considerable length of time before the test was made. This would indicate the presence of some colloid which prevented the deposit of free copper oxide. The precipitate in the tubes containing the algal extract showed that whether or not the viscous nature of the extract retarded amylolytic action, there were no colloids or other substances present which prevented a free deposit of the copper oxide.

Conclusion and summary

1. There is present in the red algae a diastase which will digest the starch of higher plants.

2. The manner of action of this enzyme indicates that it is at least partially composed of a translocation diastase.

3. The diastase of the red algae, like that of the higher plants, is probably not composed of a single enzyme, but of a series of amylases and dextrinases.

4. Judging by the action of the algal extract upon corn starch, the diastase is a rather slow-working enzyme.

5. The series of digestion processes resulting from the application of the algal diastase to corn starch would indicate that the substance composing the grains of the red algae is very similar to that of the starch grains of higher plants.

These experiments were begun at Woods Hole, Massachusetts, and completed at the University of Wisconsin. The writer wishes to take this opportunity to extend his thanks to Professor B. M. DUGGAR, at whose suggestion and under whose direction the work was begun, and also to Professor R. A. HARPER and Professor W. G. MARQUETTE for valuable assistance and helpful suggestion.

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THE MALE GAMETOPHYTE OF ABIES

A. H. Hutchinson

(WITH FIFTEEN FIGURES)

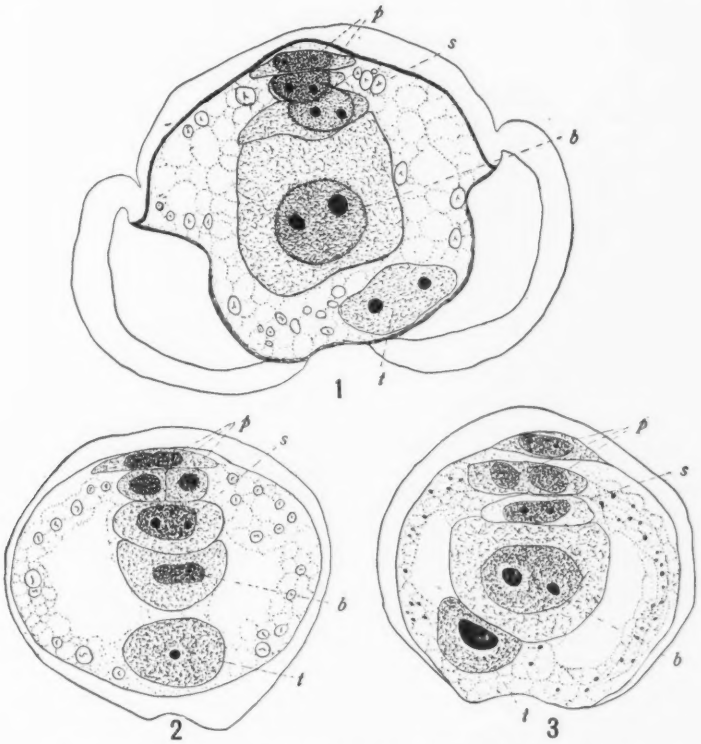
In a study of *Abies balsamea* as a type of the Coniferae, a number of irregularities in the contents of the pollen grain have been observed. Since the material used was collected from different trees and in different years, these irregularities cannot be regarded as abnormalities; and since further examination showed that similar conditions prevail in at least two other species of *Abies*, these observations have been thought worthy of record.

For the material I am indebted to Professor R. Wilson Smith. *A. balsamea* was collected in Ontario; *A. Veitchii* and *A. brachyphylla* were obtained through the courtesy of the authorities of the Royal Gardens at Kew.

The pollen grain of *Abies balsamea* is very large, its diameter being about twice that of *Pinus*. At the time of shedding the normal grain contains two prothallial cells, a stalk cell, a body cell, and a tube nucleus, imbedded in a vacuolated protoplasmic mass which contains numerous starch grains (fig. 1). The nucleus of the body cell is surrounded by dense protoplasm and a cell membrane. The tube nucleus is large and usually compressed; in some instances the pressure, resulting from the extraordinary growth of the cells of the gametophyte, is insufficient to force it from its polar position to a lateral one. The stalk cell is much smaller than the body cell, and quite frequently is compressed into a cavity at the prothallial end of the latter. In *A. Veitchii* and *A. brachyphylla* the prothallial cells are very much flattened at this stage; but in *A. balsamea* it is not uncommon to find both prothallial cells retaining the characteristic nuclear structure. The size and permanence, however, varies with the individual as well as with the species.

The material examined does not show stages earlier than the division of the central cell into generative cell and tube nucleus. Peculiarities in this division are described later. The periclinal

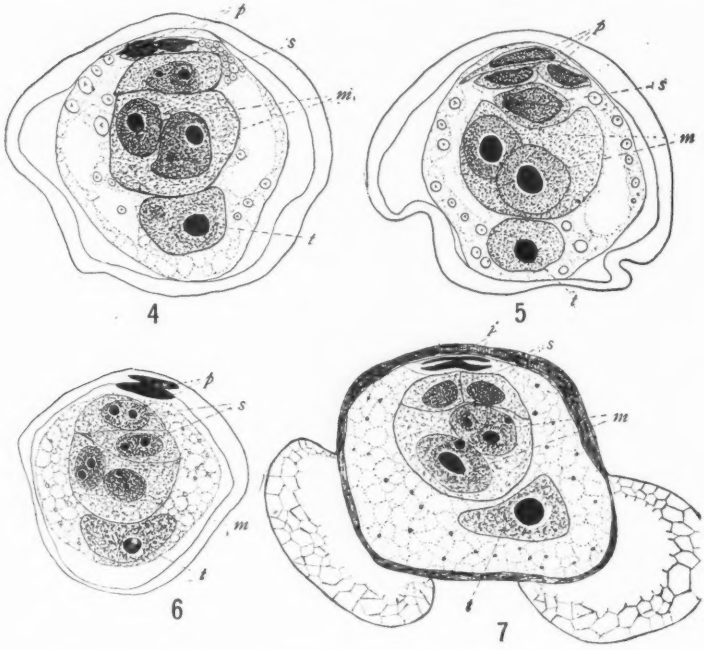
division into stalk and body cells takes place six or seven days before pollination. At this time the prothallial cells are at their maximum size.



FIGS. 1–3.—*Abies balsamea:* fig. 1, ripe pollen grain; figs. 2, 3, at time of shedding, showing divisions of prothallial cells; *p*, prothallial cells; *b*, body cell; *s*, stalk cell; *t*, tube nucleus; all ×610.

Frequent variations in the number of prothallial cells occur. The maximum number is four (figs. 2 and 4), but three are of more frequent occurrence (figs. 3 and 5). The division, as indicated by the position of the daughter cells, is anticlinal, either in or at right angles to the plane of the wings, hence they are best seen in a polar view. The presence of two derivatives of a prothallial cell, wher

they lie one over the other, is easily overlooked. It is difficult, therefore, to determine the proportion of pollen grains in which there are more than two. In *A. balsamea* about 8 per cent show a division of one or both of the original prothallial cells; in *A. Veitchii* and *A. brachyphylla* the number is smaller. In some cases



FIGS. 4–7.—Figs. 4, 5, *A. balsamea* at time of shedding, showing divisions of prothallial cells and division of body nucleus into male nuclei (*m*); fig. 5 shows nuclear division in stalk cell; figs. 6, 7, *A. Veitchii*, showing two male nuclei, two derivatives of stalk cell, and prothallial cells much flattened; lettering as in the last; all ×610.

the separating wall, if formed, becomes obliterated and the appearance is that of nuclear division only (fig. 2).

In approximately 10 per cent of the gametophytes the nucleus of the body cell divides before pollination into male nuclei. These are large and well developed, and imbedded in a common mass of protoplasm which is inclosed by the wall of the former body cell.

MIYAKE describes two male nuclei which enter the archegonium, and the one which fuses with the egg nucleus is said to be the larger. Before pollination, no difference in the size of structure could be detected (figs. 4 and 7), and MIYAKE's drawings show little or no difference in the pollen tube stage.

Occasionally the stalk cell also divides to form two derivative cells. The division is sometimes anticlinal (fig. 7), and sometimes periclinal (figs. 5 and 6). When this division takes place, the resulting nuclei frequently become uniformly granular and gradually degenerate. In this case also the separating walls are not always present (fig. 5) and the appearance is suggestive of amitosis.

There may be as many as four derivatives of the generative cell. In order to ascertain how many of the nuclei enter the egg at fertilization, an examination of the archegonium was made. Frequently there are four supernumerary nuclei at the micropylar end of archegonium after fertilization and during the proembryo stage (fig. 8). These in all probability are the second male nucleus, the two derivatives of the stalk cell, and the tube nucleus. MIYAKE states that these nuclei have the power of division. An examination of pollen grains lodged in the micropyle shows that while the other contents have passed into the pollen tube, the prothallial cells do not escape, but are retained by the intine in which they are imbedded.

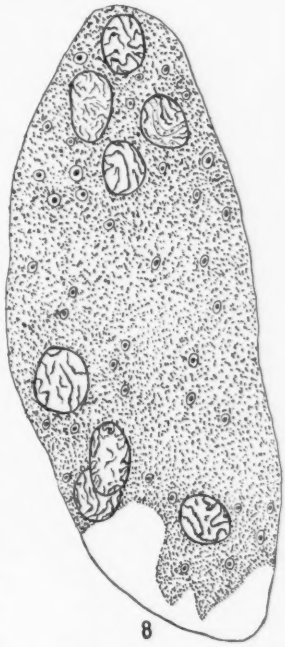
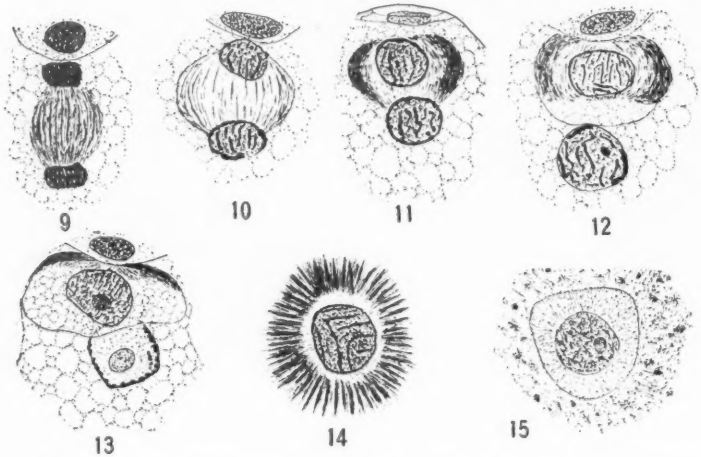


FIG. 8.—*A. balsamea:* embryo sac with four supernumerary nuclei at micropylar end, and first four nuclei of proembryo; ×610.

The division of the generative cell in *Abies* may be an adaptation to the rapid succession of events between pollination and fertilization: about 4 weeks in *A. balsamea* and 13 weeks in *Pinus*. *Picea*

excelsa (POLLOCK) is similar in this respect. The division of the stalk cell cannot be so regarded; it may represent ancestral conditions. If so, it indicates a survival in *Abies* of a more ancient type of gametophyte than has been reported in any other of the Abietineae. Further, as the presence or absence of prothallial cells is a rather constant character of the different groups of Coniferae, and thus may have a phylogenetic significance, the presence of several prothallial cells may suggest a connection of the abietin-



FIGS. 9–15.—*A. balsamea* ten days before shedding: figs. 9–13, successive stages in telophase of mitosis of central cell; the relative positions are indicated by that of the prothallial cell; fig. 14, polar view of stage shown in fig. 13; fig. 15, cross-section of generative cell below central line during stage shown in fig. 13, or just after formation of cell membrane; all ×926.

ean and araucarian lines; which connection is also indicated by evidence accumulating from other sources.

Certain peculiarities in the division of the central cell to form the generative cell and tube nucleus have been mentioned. Attention was drawn to these by the very conspicuous radiating strands which surround the generative nucleus, as seen in a polar view (fig. 14). Further investigation shows these to be derived from the spindle, and that they take part in the formation of the cell membrane.

In the late telophase no well defined cell plate is formed, although thickenings of the fibers may occur (fig. 9). The central fibers of the spindle disappear, either ceasing to be differentiated by the stain, or, as is more probable, they move out and become peripheral (fig. 10). Gradually the spindle widens and moves away from the tube nucleus, thereby inclosing the generative cell in the form of a hollow globe (figs. 10 and 11). The fibers continue to move upward until they come in contact with the wall of the second prothallial cell (figs. 12 and 13). Meanwhile the cell membrane is formed in the position occupied by the peripheral fibers. When the wall is complete the fibers have disappeared. Although derived from the spindle, the cell wall does not originate from a cell plate, but from the fibers after they have surrounded the generative nucleus. Whether or not the fibers fuse laterally is difficult to determine; that they are very numerous and form a dense, almost continuous, envelope about the generative nucleus is shown in a polar view (fig. 14). The definite cell membrane which is formed is best shown in cross-section. Fig. 15 represents such a section of a cell at the stage shown in fig. 13.

These peculiarities may perhaps be accounted for by the fact that the division is internal, and only one of the resulting nuclei is inclosed by a wall; hence this method of delimiting the cellular protoplasm. One is reminded of the formation of the plasma membrane about the ascospore of *Phyllactinia* as described by HARPER.

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CURRENT LITERATURE

BOOK REVIEWS

Parasitic fungi

A book treating of the fungi which cause plant diseases, which limits itself to the diseases already known in the United States and to those which, known elsewhere, seem likely to invade this country, ought to be received gratefully by the American student of plant pathology. In his new book, STEVENS[1] gives keys and descriptions of the fungi which cause plant diseases and also of such saprophytic forms as are obviously closely related to the parasitic forms. But he does more than give a taxonomic account of the parasitic forms, since he presents the results of cytological work, usually with the original figures, in all those groups in which extensive cytological studies have appeared. As would be expected, he also goes into details of culture methods in such groups as the rusts, where an elaborate method of procedure has been evolved by specialists.

Besides the Introduction, which treats of the nature of fungi and their relation to other thallophytes, the book comprises three divisions. The first division treats of Myxomycetes, the second of Schizomycetes. There follows a bibliography of 210 titles relating to the Introduction, Myxomycetes, and Schizomycetes. The third division of the book, which treats of Eumycetes, is divided into a portion on Phycomycetes with a bibliography of 149 titles, one on Ascomycetes with 344 references, one on Basidiomycetes with 345 references, while the fourth portion on Fungi Imperfecti is followed by a list of 459 references. In addition, there are 64 books and 12 periodicals which are listed as "some of the most useful." In connection with so extensive a bibliography it is to be lamented that citations are not made in full so as to include the titles of papers. Such a list as STEVENS publishes, in which appears an author's name, a volume reference (abbreviated as much as possible), a first page, and a date, might be quite satisfactory had we reached the day of perfect proofreading, when each word and figure would stand as it should, and it would not be necessary to turn back to the index of the volume indicated to see whether it was merely the page reference that was wrong or whether the volume number was at fault. Nor can we say that up to this moment we have found the proofreading of these bibliographies imperfect, but a number of very obvious slips in the text would indicate that this might be possible. Thus, on page 72, *Cladochytrium*, after being listed in the key under *intracellular* forms, is described as a genus containing about ten *intercellular* parasites; and on page

[1] STEVENS, F. L., The fungi which cause plant diseases. 8vo. pp. viii+754. *figs. 449.* New York: The Macmillan Co. 1913. $4.00.

75, it is said of *Dictyuchus:* "This genus of the Saprolegniaceae contains the only parasite genus in the first two families." Turning then to the end of the book, we find sprodochium for sporodochium on page 664, "synnema or corymium" for *coremium* on page 565, while *synema* appears on page 694 of the glossary, and on page 443 Merasmieae appears instead of Marasmieae.

In several points STEVENS helps to overcome inconsistencies which are common in works on parasitic fungi. Striking among these is his listing of the three groups of Fungi Imperfecti (the group which is characterized chiefly by the imperfection of our knowledge of them) in the three orders Sphaeropsidales, Melanconiales, and Moniliales, rather than Sphaeropsidales, Melanconiales, and Hyphomycetes. In this connection it is unfortunate that he should speak of the types of *fructification* displayed by Fungi Imperfecti as pycnidia, acervuli, and hyphae, in spite of the fact that the definition of hypha in the glossary is "the thread-like *vegetative* part of a fungus."

Altogether this book, with its concise, clear keys of parasitic fungi, which aims to give at least one illustration as well as description of each genus of economic importance in the United States, and which has a comprehensive bibliography, must prove a stimulus to the student of plant pathology.—WANDA M. PFEIFFER.

NOTES FOR STUDENTS

Current taxonomic literature.—H. ANDRES (Verhandl. Bot. Ver. Prov. Brandbg. **54**:218–227. 1913) has published two species of *Pyrola*, one being from the state of Washington, and (Oesterreich. Bot. Zeitschr. **63**:68–75. 1913) three species are added from the Pacific Coast. The same author (Allg. Bot. Zeitschr. **19**:81–86. 1913) in a closing article on the Pyrolaceae includes the description of a new species (*P. cordata*) from Ontario, Canada.—H. H. BARTLETT (Rhodora **15**:81–85. 1913) in continuation of systematic studies on *Oenothera* has published jointly with G. F. ATKINSON two new species of this genus from New York.—O. BECCARI (Webbia **4**:143–240. 1913) under the title "Contributi alla conoscenza delle Palme" has published the results of further studies of the palms, describes a new genus (*Jubaeopsis*) from Central Africa, and gives a revision of the genus *Pritchardia* which includes several new species from the Hawaiian Islands.—G. BITTER (Rep. Sp. Nov. **12**:49–90, 136–162. *pls. 1, 2.* 1913) has published upward of 50 new species and several varieties of *Solanum* mostly from America.—S. F. BLAKE (Rhodora **15**:86–88. 1913) records two new forms of *Ophioglossum vulgatum* from eastern North America, and (*ibid.* 153–168) under the title of "Six weeks' botanizing in Vermont. I. Notes on the plants of the Burlington region" presents the results of a study of the plants collected in the Champlain Valley of Vermont in 1911, adding several species not hitherto recorded from the Burlington region and describes a number of new varieties and forms.—C. BÖRNER (Abh. Nat. Ver. Bremen **21**:245–282. 1913) under the title "Botanisch-systematische Notizen" has

proposed several new generic names for somewhat aberrant or habitally distinct forms of well known genera. The names are numerous and attention is called merely to their place of publication.—E. BRAINERD (Bull. Torr. Bot. Club **40**:249–260. *pls. 15–17.* 1913) describes four new hybrid violets; and (Rhodora **15**:106–111. *pl. 104.* 1913) in continuation of his studies on the violets presents a discussion on the Old World *Viola arenaria* DC. in relation to its American ally and characterizes a new variety, namely, *V. adunca* var. *glabra.* The same author (*ibid.* 112–115) under "Notes on new or rare violets of Northwestern America" describes a new variety of *V. cucullata* Ait. and adds several new hybrid combinations.—A. BRAND (Ann. Conserv. and Jard. Bot. Genève **15** and **16**:322–342. 1913) in continuation of studies on the Polemoniaceae records further information on this family and describes several new species and varieties from western United States, and (*ibid.* 343–344) publishes two new species of *Symplocos* from America.—T. S. BRANDEGEE (Univ. Calif. Pub. Botany **4**:375–388. 1913) has published 35 new species, based on collections made in Mexico by Dr. C. A. PURPUS in 1912.—N. L. BRITTON (Torreya **13**:215–217. 1913) describes 4 new species of Cyperaceae from the West Indies.—N. L. BRITTON and J. N. ROSE (Contr. U.S. Nat. Herb. **16**:239–242. *pls. 66–73.* 1913) in continuation of their studies in the Cactaceae have described from living specimens 7 new species from Mexico and Central America. The same authors (*ibid.* 255–262. *pls. 78–84*) under the title "The genus Epiphyllum and its allies" present a systematic treatment of *Epiphyllum*, to which *Phyllocactus* Link is referred as a synonym, and the immediately allied genera, as follows: *Epiphyllum* (28), *Disocactus* (2), *Zygocactus* (3), *Schlumbergera* (2), *Wittia* (3), and *Epiphyllanthus* (1); *Eccremocactus* and *Strophocactus*, each represented by a single species, are proposed as new generic types. —L. BUSCALIONI and G. MUSCATELLO (Malpighia **25**:187–250. 1912) under the title "Studio monografico sulle specie americane del gen. *Saurauia* Willd." give a synoptical revision of the genus and include descriptions of two new species from South America, and (*ibid.* 389–436. *pls. 7, 8*) in continuation of these studies add two more species of *Saurauia* from Mexico.—F. E. CLEMENTS, C. O. ROSENDAHL, and F. K. BUTTERS (Geol. and Nat. Hist. Surv. Minn., Minn. Bot. Studies. pp. ix+59. 1913) have issued the third edition of their "Guide to the spring flowers of Minnesota, field and garden." —O. F. COOK (Contr. U.S. Nat. Herb. **16**:243–254. *pls. 74–77.* 1913) presents a discussion of the false date palm (*Pseudophoenix Sargentii* Wendl.) and creates for it an independent family, namely *Pseudophoenicaceae.* In an accompanying key to the families of American palms the new family is placed between the *Ceroxylaceae* and the *Cocaceae.*—L. DAMAZIO (Broteria Ser. Bot. **11**:51–53. 1913) describes and illustrates a new species of *Cassia* (*C. itaculumiensis*) from Brazil.—H. DIEDICKE (Ann. Mycol. **11**:172–184. 1913) under the title "Die Leptostromaceen" describes two new genera, namely, *Pycnothyrium* found on leaves of *Mercurialis perennis* and *Thyriostroma* on *Pteris aquilina.*—A. D. E. ELMER (Leafl. Phil. Bot. **5**:1589–1750. 1913) in cooperation with several

specialists has issued articles 78–92 inclusive of the *Leaflets*. About 120 new species and varieties of Philippine plants are described.—F. FEDDE (Rep. Sp. Nov. **12**:278–279. 1913) records a new species and variety of *Corydalis* from Idaho.—M. L. FERNALD (Rhodora **15**:74–78, 92, 93, 168, 169. 1913) has published new varieties in *Bidens*, *Carex*, and *Betula*.—M. L. FERNALD and K. M. WIEGAND (*ibid.* 133, 134) characterize two new varieties of *Carex* from Newfoundland and (*ibid.* 135, 136) a new form of *Calamagrostis Pickeringii* Gray.—C. N. FORBES (Occ. Papers Bern. Pau. Bishop Mus. Eth. and Nat. Hist. **5**:3–26. 1913) places on record notes concerning the flora of the Hawaiian Islands and describes a new species of *Euphorbia* (*E. Stokesii*).—E. GADECEAU and O. STAPF (Rev. Hort. Paris **85**:422–426. 1913) describe and illustrate a new species of *Mandevillea* (*M. Tweedieana*) indigenous to South America.—L. GAIN (Deuxième Expédition Antarctique Française, 1908–1910, commandée par le J. CHARCOT, pp. 218. *pls. 1–8.* 1912) records the results of a study of the algae secured on the expedition and describes several species new to science.—H. A. GLEASON (Bull. Torr. Bot. Club **40**:305–332. 1913) in continuation of studies on the *Vernonieae* has published several new species and gives a synopsis of the group as represented in the West Indies.—A. GRIFFINI (Atti Soc. Ital. Sc. Nat. Milano **52**:61–104. 1913) under the title "Sopra alcuni Grillacridi e Stenopelmatidi della collezioni Pantel" includes the description of a new genus (*Paterdecolyus*) from India. The new genus is related to *Anabropsis*.—D. GRIFFITH (Monatsschrift für Kakteenkunde **23**:130–140. 1913) describes several new species of *Opuntia* and a new *Nopalea* from Southwestern United States and Mexico.—H. GROSS (Bull. Geogr. Bot. **23**:7–32. 1913) under the title "Remarques sur les Polygonées de l'Asie orientale" presents a synopsis of the genera of this family, as represented in eastern China, describes several new species and one new genus (*Pleuropteropyrum*).—H. E. HASSE (Contr. U.S. Nat. Herb. **17**:1–132. 1913) has published a "Lichen flora of Southern California." The region covered by the author is that portion of California south of the 36° parallel or about one-third of the state. Five families of lichens are recognized embracing 60 genera and approximately 360 species.—E. HASSLER (Rep. Sp. Nov. **12**:201, 202, 249–278. 1913) describes about 75 new species, belonging mostly to the Apocynaceae, Malvaceae, and Onagraceae, from Argentina and Paraguay. One new genus is characterized, namely, *Casimirella* of the Icacinaceae.—A. HEIMERL (Oesterr. Bot. Zeitschr. **63**:279–290. 1913) under the title "Die Nyctaginaceen-Gattungen *Calpidia* und *Rockia*" revives *Calpidia* Thour. and proposes a new genus (*Rockia*) based on *Pisonia sandwichensis* Hillebr. The same author (*ibid.* 353–356) has published a new species of *Selinocarpus* (*S. Purpusianus*) from Mexico.—A. A. HELLER (*Muhlenbergia* **9**:60–65. 1913) in an article entitled "Acmispon in California" recognizes 6 species of this genus, 4 of which are described as new to science. The same author (*ibid.* 67, 68) makes 23 new combinations in the Leguminosae, Onagraceae, and Ericaceae.—G. HINZE (Ber. Deutsch. Bot. Gesells. **31**:189–202. *pl. 9.* 1913) under the title "Beiträge zur Kenntnis der

farblosen Schwefelbakterien" proposes a new genus (*Thiovulum*) from the Gulf of Naples.—A. KORSCHIKOFF (*ibid.* 174–183. *pl. 8.* 1913) describes and illustrates a new genus (*Spermatozopsis*) of the Volvocales.—B. KOSO-POLJANSKY (Jour. Russe de Bot. no. 1–2. pp. 1–10. *pls. 1–5.* 1913) under the title "Species Umbelliferarum minus cognitae" has published several new species of Umbelliferae and includes a new genus (*Glochidopleurum*) based on *Bupleurum Sintenisii* Aschers. and Graeb.—F. KRÄNZLIN (Ann. K.K. Naturhist. Hofmus. Wien **27**:109–112. 1913) has published 5 new species of *Spiranthes* from South America.—K. KRAUSE (Smith. Misc. Coll. **61**: no. 16. p. 1. 1913) describes a new species of *Esenbeckia* (*E. Pittieri*) from Colombia.—G. KÜKENTHAL (Rep. Sp. Nov. **12**:91–95. 1913) under the title "Cyperaceae novae III" has published several new species and varieties including 4 from South America.—C. LAUTERBACH (Bot. Jahrb. **50**:1–170. 1913) in cooperation with certain specialists has issued the second article under the general title "Beiträge zur Flora von Papuasien." Several species new to science are recorded and the following new genera are proposed: *Mischocodon* Radlk. of the Sapindaceae, *Astelma* Schltr. of the Asclepiadaceae, *Ancylacanthus* and *Jadunia* Lindau of the Acanthaceae.—R. LAUTERBORN (Allg. Bot. Zeitschr. **19**:97–100. 1913) under the title "Zur Kenntnis einiger sapropelischer Schizomyceten" characterizes the following new genera: *Pelodictyon*, *Schmidlea*, *Pelogloea*, and *Peloploca*.—H. LÉVEILLÉ (Rep. Sp. Nov. **12**:99–103. 1913) has published several new species of flowering plants from Asia and includes a new genus (*Bodinieriella*) of the Ericaceae.—G. LINDAU (Ber. Deutsch. Bot. Gesells. **31**:243–248. *pl. 11.* 1913) publishes an account of a new fungus to which he gives the name *Medusomyces Gisevii*. The new genus is related to *Mycoderma*.—T. LOESENER (Rep. Sp. Nov. **12**:217–244. 1913) in cooperation with several specialists under the heading "Mexikanische und zentralamerikanische Novitäten IV" has published several new species of flowering plants. —Fr. MARIE-VICTORIN (Le Naturaliste Canadien **39**:177–189. 1913) under "Notes sur deux cas d'hybridisme naturel" treats *Nymphaea rubrodisca* (Morong) Greene as a hybrid between *N. americana* (Prov.) Miller and Standley and *N. microphylla* Pers., and records a new hybrid between *Lysimachia terrestris* (L.) B.S.P. and *L. thyrsiflora* L.—A. MAUBLAN (Bol. Minist. Agr. Ind. and Com. Rio de Janeiro **2**:126–130. 1913) in a paper entitled "Uma molestia do mamoeiro" describes and illustrates a new fungus (*Sphaerella Caricae*) found on leaves of the pawpaw (*Carica Papaya* L.) and proposes a new generic name (*Asperisporium*) based on *Fusicladium Peucedani* Ell. & Holw.—W. R. MAXON (Smiths. Misc. Coll. **61**: no. 4. pp. 1–5. *pls. 1, 2.* 1913) describes and illustrates a new genus of ferns (*Saffordia*) from Peru. The same author (Contr. U.S. Nat. Herb. **17**:133–179. *pls. 1–10.* 1913) under the title "Studies of tropical American ferns no. 4" has published a paper embodying results of a continued study of ferns and fern-allies, recording important data and describing new species in *Asplenium*, *Dicksonia*, *Odontosoria*, *Bommeria*, *Hemionitis*, *Lycopodium*, and *Cyathea*.—N. NAOUMOFF (Bull. Soc. Mycol.

France **29**:273–278. *pl. 13.* 1913) under the heading "Matériaux pour la flore mycologique de la Russie" includes the description of a new genus (*Rhodoseptoria*) found on leaves and fruit of *Prunus*.—B. Němec (Rozpravy Ceské Akademie **21**:1–16. *pls. 1, 2.* 1912) describes and illustrates a new genus and species (*Anisomyxa Plantaginis*) parasitic on *Plantago lanceolata*.—J. A. Nieuwland (Am. Mid. Nat. **3**:85–91. *pl. 2.* 1913) has published a new species of violet (*Viola candidula*) from Michigan.—S. B. Parish (Bot. Gaz. **55**:300–313. 1913) under the title of "California Paroselas" recognizes 9 species and several varieties of this genus indigenous to California. The same author (Muhlenbergia **9**:57–59. 1913) in an article entitled "Additions to the known flora of southern California" places on record important data concerning that flora and describes a new species of *Atriplex* (*A. saltonensis*) from the Colorado Desert.—F. W. Pennell (Bull. Torr. Bot. Club **40**:401–439. 1913) gives a synoptical revision of the *Agalinanae*, recognizing four genera, namely *Macranthera, Afzelia, Aureolaria*, and *Agalinis*. Descriptions of several new species are included.—C. A. Picquenard (Trav. du Lab. de Concarneau **4**: fasc. 3. pp. 1–5. *pl. 1.* 1912) has proposed a new genus (*Guerinea*) based on *Hapalidium callithamnoides* Crouan.—R. Pilger (Bot. Jahrb. **50**:171–287. 1913) in continuation of monographic studies of the Plantaginaceae gives a detailed consideration of the section *Novorobis* of *Plantago*, recognizing 50 species of which nearly one-third are new to science. The same author (Rep. Sp. Nov. **12**:304–308. 1913) has published 6 new species of grasses from Patagonia and Tierra del Fuego.—L. Quehl (Monats. für Kakteenkunde **22**:42. 1913) describes a new species of *Mamillaria* (*M. echinoidea*) from Mexico.—H. Rehm (Ann. Mycol. **11**:166–171. 1913) characterizes a new ascomycetous genus (*Durandia*) based on *Sphaerographium Fraxini* (Peck) Sacc.—H. N. Ridley (Jour. Bot. **51**:201, 202. *pl. 527.* 1913) has published a new genus (*Aridarum*) of the Araceae from Borneo.– W. J. Robinson (Bull. Torr. Bot. Club **40**:193–228. *pls. 9–12.* 1913) under the title of "A taxonomic study of the Pteridophyta of the Hawaiian Islands III" presents a synoptical treatment of several genera and includes descriptions of new species in *Polypodium* and *Asplenium*.—R. A. Rolfe (Bot. Mag. *t. 8514.* 1913) describes and illustrates a new orchid (*Catasetum microglossum*) from Peru.—J. N. Rose (Smiths. Misc. Coll. **61**: no. 12. pp. 1, 2. *pl. 1.* 1913) describes and illustrates a new poplar (*Populus MacDougalii*) from the Salton Basin, California.—P. A. Rydberg (Bull. Torr. Bot. Club **40**:461–485. 1913) under the title "Studies on the Rocky Mountain flora XXIX" describes several new species of Sympetalae.—R. Schlechter (Rep. Sp. Nov. **12**:104–109, 202–206, 212–216. 1913) in continuation of his work on orchids has published upward of 20 new species from tropical America. One new genus (*Ischnogyne*) from the mountains of Setschuan, China, is included.—C. W. Shannon (Okl. Geol. Surv. no. 4. pp. 41. 1913) has issued a list of the trees and shrubs of Oklahoma.—J. K. Small (Torreya **13**:77. 1913) describes a new species of *Malpighia* (*M. Harrisii*) from Jamaica.—J. D. Smith (Bot. Gaz. **55**:431–438. 1913) presents

his 36th paper, as a result of continued study on the flora of Central America. The article includes descriptions of 12 species of flowering plants new to science. —J. D. SMITH and J. N. ROSE (Contr. U.S. Nat. Herb. **16**:287–298. 1913) have published a "Monograph of the Hauyeae and Gongylocarpeae, tribes of Onagraceae." The study embraces 4 genera and 14 species; two new generic names are proposed, namely, *Xylonagra*, based on *Oenothera arborea* Kellogg, and *Burragea*, based on *Gaura fruticulosa* Benth.—W. W. SMITH (Rec. Bot. Surv. India **4**:324–431. 1913) records the results of a botanical survey of Southeast Sikkim, India, lists 925 species, and describes a new genus (*Paroxygraphis*) of the Ranunculaceae.—C. SPEGAZZINI (Ann. Mus. Nac. Buenos Aires **23**:167–244. 1912) in an article on the Laboulbeniaceae of Argentina has published several new species and proposes two new genera, namely, *Cochliomyces* and *Laboulbeniella*. The same author (*ibid.* 1–146) under the title "Mycetes Argentinenses" continues the enumeration of the Mycetes of Argentina, adds several species new to science, and proposes the following new genera: *Eudimeriolum*, *Winteromyces*, *Trichospermella*, *Dasysphaeria*, *Criserosphaeria*, *Hormopeltis*, *Polhysterium*, *Symphaeophyma*, *Apiosporella*, *Ectosticta*, *Dasysticta*, *Dasyprena*, *Phaeopolynema*, and *Phaeolabrella*.—A. STEWARD (Proc. Calif. Acad. Sci. **1**:431–446. 1912) records the lichens found on the Expedition of the California Academy of Sciences to the Galapagos Islands in 1905–1906. Sixteen species were found which were not before reported from the islands.—H. and P. SYDOW (Ann. Mycol. **11**:93–118. 1913) have published several new species of fungi from northern Japan and characterize a new genus (*Miyagia*) of the Pucciniaceae found on leaves of *Anaphalis margaritacea*. The same authors (*ibid.* 254–271) under the title "Novae fungorum species X" have published several species new to science and propose the following new genera: *Aithaloderma*, *Schizochora*, *Cyclodothis*, and *Diedickea* from the Philippine Islands, *Astrosphaeriella* and *Coccidophthora* from Japan, and *Nematostigma* from South Africa.—C. TORREND (Broteria, Ser. Bot. **11**:73–98. 1913) under the title "Les Basidiomycetes des environs de Lisbonne et de la région de S. Fiel (Beira Baixa)" includes the descriptions of several new species and proposes one new genus, namely, *Lycoperdellon*, based on *Lycogala Torrendii* Bres.—I. URBAN (Bot. Jahrb. **50**: Beibl. 111. pp. 1–108. 1913) under the title "Plantae novae andinae imprimis Weberbauerianae VI" in cooperation with several specialists has published an important paper on the Andean flora. About 125 species new to science are described.—H. F. WERNHAM (Jour. Bot. **51**:218–221. 1913) has published 11 new species of Rubiaceae from tropical America. —R. S. WILLIAMS (Bryologist **16**:36–39. *pl. 4.* 1913) reports *Brachymenium macrocarpum* Card. from Florida and describes a new species of *Funaria* (*F. rubiginosa*) from Montana.—J. M. GREENMAN.

Metabolism of fungi.—Believing that methods based on a determination of the yield, or of the economic or the respiratory coefficients do not give a satisfactory quantitative representation of the manner of utilization of carbon

compounds by fungi, WATERMAN[2] has applied the conceptions of "plastic equivalent" and "respiratory equivalent" to the study of the carbon metabolism of *Aspergillus niger*. The "plastic equivalent" for carbon is defined as that fraction or percentage of the consumed carbon which at any given moment is contained in the substance of the organism. Similarly, the "respiratory equivalent" is the percentage which has up to that moment reappeared as carbon dioxide. Experimentally these relations are obtained by determinations of the carbon given off in respiration, the quantity fixed in the body of the fungus, and the total quantity that has been consumed.

The author has studied from this point of view the assimilation of glucose, levulose, mannose, and a number of organic acids. As a rule, however, only the plastic equivalents were determined, the respiratory equivalent having been determined only for succinic acid. The results show, apparently to the mild surprise of the author, that the plastic equivalent is high during the early stages of growth, and falls with increasing age of the culture; while the greater part of the carbon nutrient disappears during the first few days of growth. From these observations he arrives at his main conclusion, that the temporary accumulation of carbon in the fungus is due to the formation of an intermediate product which he finds to be glycogen. This view, of course, presents nothing novel, for it is well known that in the presence of an excess of food, reserve materials, which in fungi mostly take the form of glycogen, are stored in the plant body, and that respiratory activity, decreasing the "plastic equivalent," continues at the expense of reserve materials and even at the expense of proteids when the external food supply has been exhausted. All such substances must in this sense be regarded as intermediate products. In this connection it may be pointed out that from the discrepancy between the quantity of carbon dioxide developed by a fermenting mixture and that which should have been developed according to the quantity of glucose (determined by change in rotation) which disappeared from the mixture, EULER and JOHANSSON[3] have recently concluded that intermediate products were first formed from the glucose in the process of fermentation. Regarding the author's method it should also be stated that SPIEKERMANN,[4] without giving special names to the ratios, determined for a species of *Penicillium* growing on glycerin the percentage of the consumed carbon fixed in the plant body, and that given off in respiration.

Finally, WATERMANN has investigated the influence of various factors in relation to the plastic equivalent. Changes in temperature and in concentration of the nutrient medium do not influence the nature but only the rate of

[2] WATERMAN, H. I., Beitrag zur Kenntnis der Kohlenstoffnahrung von *Aspergillus niger*. Folia Microbiol. **1**:422–485. 1913.

[3] EULER, H., und JOHANSSON, D., Umwandlung des Zuckers und Bildung der Kohlensäure bei der alkolischen Gärung. Zeitschr. Physiol. Chem. **76**:347–354. 1912.

[4] SPIEKERMANN, A., Die Zersetzung der Fette durch höhere Pilze. Zeitschr. Unters. Nahrungs- u. Genussmittel **23**:305–331. 1912.

metabolism. The magnitude of the plastic equivalent is to a high degree dependent on the nature of the carbon nutrient. This relation is correlated with the heats of combustion of the carbon compounds. Those having the greater caloric value give the highest plastic ratios.—H. HASSELBRING.

Bud variations and fruit markings.—This very interesting question is the subject of a paper by KRAUS,[5] who has been making studies on the effects of cross-pollination of cultivated fruits. The author calls attention to the frequent occurrence of banded or striped fruits, especially among apples. The most common explanation of this phenomenon is the secondary influence of pollen, but the author explains that this cannot be true xenia, such as occurs in corn. Correspondence with horticulturists and botanists indicated a prevailing opinion that it is due to secondary influence of pollen, though a number believed it due to bud-variation. After explaining the economic importance of the problem, the author describes his methods of work. The conclusions are as follows: "color in the pome fruits is not influenced directly in the immediate cross; new characters cannot be added by the pollen, outside the seed itself, in the immediate cross; the manifestation of color is dependent on many environmental factors; color as usually found is composed of a number of unit characters; somatic segregation may occur and by this means the several factors of color manifest themselves more or less independently (the several colors may appear as bands more or less parallel, or a band of but one color surrounded by the normal color); similar segregation may extend to any group of unit characters of which the plant is composed; segregation may extend to either fruit or leaf buds; if the latter, such variations may be propagated asexually; red in apples may consist of either a single or a complex of unit characters; at least, three reds are recognizable; somatic segregation may be of service to plant breeders as indicating the unit characters of a plant that are likely to exhibit themselves when propagated sexually; segregation generally extends to the flower bud only in apples, while in pears the shoot is frequently affected."—MEL T. COOK.

The development of chalazogams.—NAWASCHIN and FINN[6] have published a contribution in German which extends the study of *Juglans* published in Russian a year ago and already noted in this journal.[7] The principal conclusions are: that in seed plants there is a tendency to reduce the male gametes from sperms to naked nuclei; that the evolution of the pollen tube and simplification of the sperm go hand in hand; that *Juglans* and other chalazogamous plants with a well developed binucleate cell which reaches the embryo

[5] KRAUS, E. J., Bud variations in relation to fruit markings. Biennial Crop Pest and Horticultural Report for 1911 and 1912. Oregon Agric. Exp. Station. pp. 71–78.

[6] NAWASCHIN, S., and FINN, V., Zur Entwickelungsgeschichte der Chalazogamen. *Juglans regia* und *Juglans nigra*. Mém. Acad. Imp. Sci. St. Pétersbourg **31**:1–59. *pls. 1–4.* 1913.

[7] BOT. GAZ. **55**:94. 1913.

sac in fact show a condition intermediate between a well developed sperm and a naked sperm nucleus; and that this feature indicates the great age of chalazogams. These conclusions, which are practically the same as those given in the previous paper, are based upon a large amount of research and also upon a thorough discussion of the literature, in which the work of American investigators receives generous recognition. Both authors had already become identified with the subject, and no one has contributed more to our knowledge of chalazogamous plants than NAWASCHIN. Besides, as the discoverer of "double fertilization," he has made a reputation for brilliant initiative in research, while his more recent investigation of the sperm nucleus of *Lilium Martagon* entitles him to a place among the authorities in cytological matters. These facts lend weight to the conclusions. The paper deserves a careful reading by everyone who attempts to treat the phylogeny of angiosperms from a cytological standpoint. Three of the large plates are colored, and the fourth (copied from various investigators) gives a useful optical survey of pollen tube structures in various groups of gymnosperms and angiosperms.—CHARLES J. CHAMBERLAIN.

Cecidology.—Among the important foreign contributions is a purely botanical paper by BUYSSON and PIERRE,[8] in which two species occurring on *Sedum* are discussed. HOUARD[9] gives good descriptions of a number of cecidia in the Natural History Museum of Paris, restricting his discussions to the galls and not to the causes. KIEFFER[10] describes two new genera and two new species of cecidomyid galls and gall-makers from Formosa.

Among the most interesting American contributions is a very suggestive paper on seedless and malformed fruits by BROWN,[11] in which the author, after discussing malformations and russetings caused by frost, also calls attention to the fact that fruits may be abnormal as a result of no pollination or imperfect pollination combined with frost injuries. After pollination, a severe frost may interfere with the fertilization processes and affect both seed and fruit. The author also states that there is relationship between weights of seeds and fruit but does not give figures.

FELT[12] contributes a very valuable entomological study of gall midges, in which he includes keys, descriptions, and drawings of a great many species. —MEL T. COOK.

[8] BUYSSON, H. DU, et PIERRE, l'ABBE, Nouvelles cecidologiques du centre de la France. Marcellia **12**:27–35. 1913.

[9] HOUARD, C., Les collections cécidologiques du laboratoire d'entomolgie du. Museum d'histoire naturelle du Paris: Galles du Mavor. Marcellia **12**:35–41. 1913.

[10] KIEFFER, J. J., Description de deux remarquables cécidomyies de Formose. Marcellia **12**:42–44. 1913.

[11] BROWN, F. R., Seedless and malformed fruits. Biennial Crop Pests and Horticultural Report for 1911 and 1912. Oregon Agric. Exp. Station.

[12] FELT, E. P., A study of gall midges. Twenty-eighth Report of the Entomologist of the State of New York. pp. 127–226. 1913.

Parthenogenesis in Bennettites.—Parthenogenesis, or the development of an embryo from an egg without fertilization, has been demonstrated for several angiosperms, and is claimed for *Pinus pinaster* and *Gnetum Ule*. In spite of the difficulty of proving a case of parthenogenesis in living seed plants, LIGNIER[13] has given us a short paper with the rather startling title "*Bennettites Morieri* probably reproduces by parthenogenesis." One naturally looks for the evidence. Here it is. He found embryos, but no pollen grains or pollen tubes, and the tip of the nucellus was closed. Some of the sections were in series, at intervals of about 3 mm. Presumably most of the sections were not in series. When one remembers that pollen tubes have never been demonstrated in the Bennettitales and that very possibly the pollen grains may shed their sperms without the formation of a pollen tube, and that even in rather thick sections at intervals of 3 mm., more than nine-tenths of the material is missing, the evidence is not convincing. And yet, this "probable parthenogenesis" is given as a reason for the rapid disappearance of this group during the Cretaceous.—CHARLES J. CHAMBERLAIN.

A classification of conifers.—SAXTON[14] has proposed a classification of conifers based upon the great extension of knowledge of the group developed during recent years. There is no question that the current classifications are archaic, and that a more natural classification of the group is demanded. SAXTON analyzes the characters to be used in such a classification, and discusses the various attempts that have been made. He then describes, with definite characters, five families (Araucariaceae, Podocarpaceae, Pinaceae, Cupressaceae, and Taxaceae), Pinaceae having two subfamilies (Abietoideae and Sciadopitoideae) and Cupressaceae three (Cupressoideae, Callitroideae, and Sequoideae). A consideration of the phylogeny of Coniferales results in an interesting "family tree" that shows the relationships of the families and subfamilies. An interesting item of the phylogeny, at this time, is that the araucarians are represented as the first offshoot from the common stock (presumably abietinean) that arises from Cordaitales, which later gave rise to the podocarps, and then the other families.—J. M. C.

Artificial parthenogenesis in Fucus.—Parthenogenesis in various members of the Phaeophyceae has been known for some time, and unfertilized eggs of *Fucus* have been caused to divide by treating them with solutions. OVERTON[15] took exceptional care to obtain unfertilized eggs of *Fucus vesiculosus*, and each collection was divided into three lots, one of which was then fertilized, another was allowed to remain in normal sea water, while the third was treated with

[13] LIGNIER O., Le *Bennettites Morieri* (Sap. et Mar.) Lignier se reprodusait probablement par parthénogénèse. Bull. Soc. Bot. France IV. **11**:125–127. 1911.

[14] SAXTON, W. T., The classification of conifers. New Phytol. **12**:242–262. 1913.

[15] OVERTON, J. B., Artificial parthenogenesis in *Fucus*. Science **37**:841, 844. 1913.

acetic or butyric acid. The general result of the various cultures was that in the first case the sporelings developed normally; in the second, no sporelings appeared; while in the third, sporelings developed up to the 25-cell stage. The experiments were not carried farther. No cytological work was done. Since the *Fucus* plant is the *2x* generation, it would be interesting to know the chromosome situation, especially if the plants should develop up to the reproductive phase.—CHARLES J. CHAMBERLAIN.

Chromosome conjugation.—Miss FRASER,[16] in a short discussion of chromosome conjugation, cites the work of OVERTON, HARPER, DIGBY, and others to show that it is not a matter of primary importance whether parasynapsis or telosynapsis takes place, and that they need not be mutually exclusive. The sexual nuclei may fuse at once upon fertilization, or not until the division of the oospore in other cases (Pinaceae); while in the extreme case of the rusts they remain distinct until just before meiosis. In like manner the attraction between the homologous chromosomes may bring about their conjugation as soon as the nuclei fuse, or in other cases later, even as late as the formation of the gemini of maturation. The suggestion is made that the clearest cases of Mendelian inheritance will perhaps be found to be those correlated with a late association of the chromosomes in pairs.—L. W. SHARP.

Flora of Boulder.—DANIELS[17] has made a study of the vascular flora of Boulder, Colo., and vicinity, a most interesting mountain region. An introduction (48 pp.) describes the physiography, the climate and rainfall, and the zones of vegetation. The zones given are Campestres, Mensales, Submontanae, Montanae, Subalpestres, and Alpestres, each with numerous subdivisions. The list of plants (211 pp.) includes 1225 numbers in 486 genera, with a statement as to the habitat of each species. A number of new combinations are made, and new species described in *Acomastylis* (*Geum*), *Prunus*, *Vitis*, *Castilleja*, and *Grindelia*. One of the unique features of the list is that a popular name is given for each species. When this reaches such a stage as "filiform toad-flax-leaved painted cup," it is probable that it ceases to be useful.—J. M. C.

Graft hybrids.—Miss HUME[18] has investigated three graft hybrids for connecting protoplasmic threads. The "periclinal chimaeras" used were *Cytisus Adami*, *Solanum tubingense*, and *S. Kolreuterianum*, since in these the epidermis is the only layer of cells belonging to the one component, and the line of demarcation between the two components is therefore a sharp one. BUDER

[16] FRASER, H. C. I., The pairing of the chromosomes. New Phytol. **11**:58–60. 1912.

[17] DANIELS, FRANCIS POTTER, The flora of Boulder, Colorado, and vicinity. Univ. Missouri Studies. Science Series **2**: no. 2. pp. xiii+311. 1911.

[18] HUME, MARGARET, On the presence of connecting threads in graft hybrids. New Phytol. **12**:216–221. 1913.

had already discovered connecting threads between the two components in *Cytisus Adami*, which Miss HUME confirmed. In *Solanum tubingense* she found them, while in the other *Solanum* she did not. These results show that genetically unrelated tissues can be joined by connecting threads, and the inference is that such threads do not arise from spindle fibers, since no nuclei of the two components have even been sisters.—J. M. C.

The embryo sac of Bellis.—The peculiar development of the antipodal region in some Compositae has long been known. In 1895 the reviewer[19] described an "antipodal oosphere" in *Aster novae-angliae*, and nearly ten years later Miss OPPERMAN[20] not only found an antipodal oosphere in *Aster undulatus*, but noted its fertilization. Recently, CARANO[21] has described a "pseudo-oosphere" in the antipodal region of *Bellis perennis*. In all these cases, the region in which the abnormal oosphere is found is greatly enlarged; in fact, it has the appearance of an embryo sac. Doubtless the conditions in the enlarged antipodal cell are about the same as in a normal embryo sac, and consequently the occasional organization of an oosphere is not so strange as we formerly supposed.—CHARLES J. CHAMBERLAIN.

Reproduction in gymnosperms and angiosperms.—Under this title ERNST[22] gives an excellent résumé of the present status of the subject. The illustrations, which are taken from the leading contributions, are well reproduced. The bibliographies are in two categories: those which treat the subject in a general way, like textbooks, and those which deal with original investigation. The title, *Hand dictionary of the sciences*, is somewhat misleading for English-speaking people, for this "dictionary" is more like an encyclopedia, consisting of several volumes, edited by a staff of specialists. OLTMANNS is the general editor of botany, and he has distributed the various topics to specialists in the various fields. All articles, like the one just mentioned, are signed.—CHARLES J. CHAMBERLAIN.

Mitosis in Oenothera.—In the somatic divisions of *Oenothera lata* GATES[23] finds the chromosomes forming from the delicate reticulum by the parallel fusion of several strands. No prochromosomes are present, and no continuous spirem is formed. The splitting of the chromosomes occurs in late prophase,

[19] CHAMBERLAIN, CHARLES J., The embryo sac of *Aster novae-angliae*. BOT. GAZ. **20**:205–212. *pls. 15, 16*. 1895.

[20] OPPERMAN, MARIE, A contribution to the life history of *Aster*. BOT. GAZ. **37**:353–362. *pls. 14, 15*. 1904.

[21] CARANO, ENRICO, Su particolari anomalie del sacco embrionale di *Bellis perennis*. Annali di Botanica **11**:435–439. *pl. 9*. 1913.

[22] ERNST, A., Fortpflanzung der Gymnospermen und Angiospermen. Abdruck aus Handwörterbuch der Naturwissenschaften **4**:227–261. *figs. 37*. 1913.

[23] GATES, R. R., Somatic mitosis in *Oenothera*. Ann. Botany **26**:993–1010. *pl. 86*. 1912.

but the split disappears temporarily before the metaphase, when it is again evident. During late prophase and metaphase the chromosomes often show a distinctly paired arrangement. In telophase there is a massing at each pole, after which the chromosomes separate and become joined by anastomoses. Considerable variation in chromosome number is shown; the usual number is 15. The nuclei occasionally show certain characters of heterotypic mitosis.—L. W. SHARP.

Poison weed.—Larkspurs have always had the reputation of being poisonous, but it seems that only in North America have they been important in causing losses of stock. MARSH and his associates[24] have investigated the poisoning due to larkspur in Colorado, and presumably the conditions are the same in other mountain cattle ranges of the West. The larkspurs are grouped as tall and low larkspurs, *Delphinium Barbeyi* representing the first group, and *D. Nelsonii* the latter. These forms cause the loss of a great number of cattle, but horses and sheep are not injured by grazing on larkspur areas; and cattle are not injured if prevented from grazing on such areas until the second week of July. The next problem is to discover the specific poison.—J. M. C.

Xenia.—Having discovered instances of xenia in wheat, BLARINGHEM[25] sought to determine whether the vigorous development of hybrid enbryo and endosperm ever causes a change in the character of the maternal tissue that surrounds them. On crossing a comparatively small wheat, known as *Triticum turgidum gentile* Al. var. Normandy, with a larger type, *Triticum vulgare lutescens* hybrid no. 126 of the Hohenheim collection, 16 hybrid seeds approaching the paternal variety in size were obtained. This phenomenon is interpreted as xenia in the original sense of the term, though it seems probable that it is simply a stretching of the pericarp due to a large hybrid embryo and endosperm.—E. M. EAST.

Artificial cell structure.—In a series of interesting experiments, W. MAGNUS[26] has produced, from paraffin, beeswax, and other substances, various structures which bear a striking resemblance to cells and tissues. The paraffin, with a melting point of about 74° C., was poured upon mercury which had been heated to 78° C. and allowed to cool at room temperature. While this is only the beginning of the investigation, the writer thinks he has already shown that through the action of purely physical forces structures can be produced which look like

[24] MARSH, C. DWIGHT, CLAWSON, A. B., and MARSH, HADLEIGH, Larkspur or "poison weed." U.S. Dept. Agric., Bur. Plant Ind., Farmer's Bull. 531. 1913.

[25] BLARINGHEM, L., L'influence du pollen visible sur l'organisme maternal; découverte de la xénie chez le blé. Bull. Soc. Bot. France **60**: 187–193. 1913.

[26] MAGNUS, WERNER, Über zellenformige Selbstdifferenzierung aus flüssiger Materie. Ber. Deutsch. Bot. Gesells. **31**: 290–303. *pl. 13.* 1913.

cells. Further work is expected to show to what extent the physical forces concerned in the living and inorganic material are identical.—CHARLES J. CHAMBERLAIN.

Ovulate flower of Gnetum.—The publication of Miss BERRIDGE's paper[27] on the ovulate strobilus of *Gnetum Gnemon*, in which she gave evidence for the conclusion that the ovule was "primitively surrounded by a whorl of male flowers," has called out a paper by LIGNIER and TISON[28] upon the same structure. They have found material that seems to indicate that the "third integument" of the ovule is a modified axis of inflorescence that bore an axillary ovule; and that occasionally an axillary group of staminate flowers is present, which indicates a connection with *Welwitschia*.—J. M. C.

Beech forest on chalk and on schist.—Comparing the English beech forests on chalk with the French on schist SKENE[29] finds that they are exactly similar ecologically, and that scarcely a member of the latter is a calcifugous plant, while scarcely a single member of the former is a calcicolous plant. Topographically there is no distinction between the two types. This leads SKENE to question the accuracy of placing the two forests in different formations according to the classification adopted by British ecologists.—GEO. D. FULLER.

A bibliography of mitosis.—A very useful list of works on meiosis and somatic mitosis in the angiosperms since 1880 has been compiled by PICARD.[30] The forms are arranged according to systematic position. Although the author has not attempted to make the citations on the individual plants exhaustive, the 300 and more citations given justify him in his belief that from the list one can obtain reference to all the literature of the subject.—L. W. SHARP.

Embryogeny of the Ranunculaceae.—In continuing his studies of the Ranunculaceae, SOUÈGES[31] has described the development of the embryo of *Ficaria ranunculoides*, including some interesting cytological details.—J. M. C.

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[27] See BOT. GAZ. **55**:172. 1913.

[28] LIGNIER, O., et TISON, A., L'ovule tritégumenté des *Gnetum* est probablement un axe d'inflorescence. Bull. Soc. Bot. France **60**:64–72. *figs. 5.* 1913.

[29] SKENE, MACGREGOR, The relation of the beech forest to edaphic factors. Jour. Ecol. **1**:94–96. 1913.

[30] PICARD, M., A bibliography of works on meiosis and somatic mitosis in the angiosperms. Bull. Torr. Bot. Club **40**:575–590. 1913.

[31] SOUÈGES, R., Recherches sur l'embryogénie des Renonculacées. Bull. Soc. Bot. France **60**:150–157. *pl. 11. figs. 288–315.* 1913.